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THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

PHILADELPHIA, PA.

THE INFLUENCE OF THE MARGINAL SENSE ORGANS ON THE RATE OF REGENERATION IN CASSIOPEA XAMACHANA

LEWIS R. CARY

Princeton University

ELEVEN FIGURES

INTRODUCTION

The previous studies on the influence of the nervous system upon regeneration have given very divergent results which can hardly be reconciled even when the fact that widely separated groups of animals were used as the material for experimentation is taken into consideration.

While on the one hand certain students of this problem (Herbst, Goldstein, Walter, Wolff) have taken the position that the nervous sytem in general, or some portion of it (sensory ganglion Herbst, Walter), exerts a stimulus necessary for the complete regeneration of normal structures; other workers have attributed less and less importance to these influences. The intermediate position that the influence of the nervous system is indirect, being exerted mainly through the controlling of motor activity is well expressed by Child ('05a) in the statement concerning anterior regeneration in Leptoplana that, "as in posterior regeneration there is a close parallelism between the rapidity, amount and completeness of anterior and lateral regeneration and the characteristic motor activity of the part concerned."

Goldfarb ('09) concludes from his experiments on newts, earthworms and planarians that "these experiments . . . . should make one cautious about accepting the view of the direct or even indirect influence of a nervous influence on regeneration."

In all these studies the point at issue has been whether or not complete regeneration of typical structures is possible in the ab-

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sence of any influences exerted through the central nervous system. An affirmative answer to this question is apparently held, by certain at least of these investigators, to settle finally the question of nervous influence without any consideration being given to the comparison of the course of the regenerative process in animals in which the nervous system was removed from the regenerating area and those in which the nervous system had been uninjured in the portion of the animal left to regenerate. In only a relatively few animals can the nerve centers be removed without bringing about the destruction of, or degenerative changes in, other intimately connected portions of the nervous system, so that this type of operation has not been frequently undertaken.

Zeleny ('07) and Stockard ('08) removed the marginal sense organs from the disk of Cassipoea xamachana to determine the influence of these structures on the rate of regeneration. Both report that there was no evidence of any regulatory influence. In Zeleny's experiments the entire margin of the disk with its sense organs was removed and the rate of regeneration in these individuals compared with others in which the bell margin and sense organs were intact. In Stockard's experiments the results obtained from specimens prepared as above described were supplemented by those obtained with individuals from one-half of which the marginal sense organs were removed while from the other half an equal amount of tissue was cut from between the sense organs. The two halves were insulated by the removal of two diametrically opposite strips of subumbrella ectoderm. In both experiments the rate of regeneration was measured inward from the periphery of a cavity in the center of the disk from which a circular piece of tissue had been removed.

In both these researches the experiments were carried out with the view of ascertaining the influence of muscular activity and thus indirectly of the nervous system on the rate of regeneration. In each case it was held that there was no constant difference in the rate of regeneration between the active and inactive individuals.

In the course of my studies, which were taken up primarily to reexamine the work of Stockard and Zeleny upon this point, I discovered the marginal sense organs influence regeneration, independent of their control of muscular activity. Such an influence of the sense organs can be accounted for either on the ground that metabolic activities, not expressed by muscular activity, are under the control of the sense organs, or that a direct trophic influence is exerted by the sense organs on the regenerating tissues. A series of determinations with the 'biometer' of the rate of CO_2 production by specimens under different experimental conditions, for which I am indebted to Dr. S. Tashiro, shows that the first of the two alternatives just mentioned offers a satisfactory explanation of the observed facts.

It is a pleasure to acknowledge my indebtedness to the authorities of the Carnegie Institution of Washington for putting at my disposal the facilities of the laboratory at Dry Tortugas, Florida, and especially to Dr. A. G. Mayer the director of the laboratory for many helpful suggestions and constant interest throughout the course of the work.

TECHNIQUE

As Mayer, Stockard and Zeleny have pointed out the disk of Cassiopea will live for an indefinite period after the removal of the oral arms and retain its full capacity for regeneration. These nearly flat circular disks with their sixteen equally spaced sense organs and with the ectodermal musculature entirely on the subumbrella surface offer exceptionally favorable material for the study of the phenomena of regeneration as they will withstand practically any type of operation.

The medusae can be procured in great numbers from the moat at Fort Jefferson at Dry Tortugas, Florida, so that specimens of any desired size can be selected for experimentation. In preparing material for my experiments specimens of about 100 mm. in diameter were chosen, the oral arms and stomach removed as soon as they were brought into the laboratory and the operations, of whichever type, performed as soon as convenient, so that in

every case the experiments were started within two hours after the medusae had been removed from their normal surroundings.

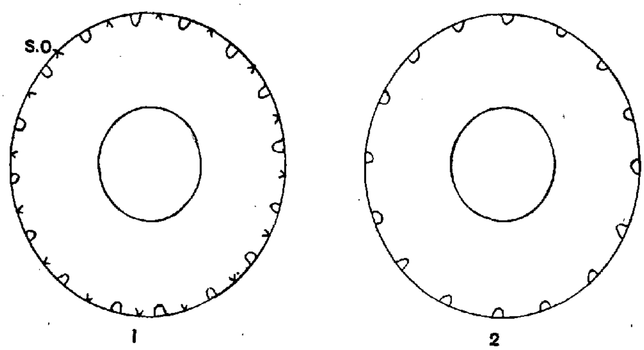
As the normal habitat of Cassiopea is in rather stagnant quiet water, the disks retain their vitality indefinitely when a single pair are kept in a medium sized battery jar of sea water. Indeed Stockard found that the benefit derived from the daily change of water was more than offset by the harmful effects of the agitation attendant upon the changing of the disks from one jar to another. Since my experiments necessitated the daily measuring of the regenerated tissue which could be done only by removing the disks from the jars and placing them upon a background of colored glass, the water was changed daily.

In specimens in which the halves were insulated it was necessary to scrape over the denuded strips at least once every forty-eight hours, as within that space of time new nerve fibers would be regenerated connecting the old nerve fibers of the insulated halves and consequently transmitting the stimulus necessary for pulsation from the sense organs of the half disk on which they were retained to the muscles of the half disk from which they had been removed.

In a few of the experiments in which regeneration was slowest, particularly some of those where the sense organs were removed from both halves of the disks, new functional sense organs were developed in the course of an experiment so that a second removal of tissue from the portion of the bell margin originally occupied by the sense organs was necessitated.

In order to determine the influence of the sense organs on the rate of regeneration the following experiments were carried out. First those in which the influence of the sense organs was removed from an entire disk (Zeleny's operation, figs. 1 and 2) or from one-half of a disk (Stockard's operation, fig. 3) by the removal of an appropriate number of sense organs and insulation of the two halves (figs. 1 and 2). Second, disks from which all but one of the sense organs were removed, with the disk either left with its subumbrella ectoderm continuous or with the halves insulated (figs. 4 and 5). Third, disks prepared according to Stockard's method were subjected to the action of anesthetics to eliminate

muscular activity. Fourth, disks prepared in the manner just mentioned were treated with solutions of oxalic acid which will destroy the sense organs without seriously injuring the muscular system or the conducting portions of the nervous system. Fifth, specimens from which all sense organs had been removed and with the halves insulated in one of which muscular activity was initiated by means of an induction shock and maintained as a circuit wave of contraction in an endless labyrinth of muscle



Figs. 1 and 2 Experiments of type 1.

Fig 1. Active disk with sense organs intact, and with pieces of tissue cut from the margin of the disk between them.

Fig. 2 Inactive disk from which all of the sense organs have been removed.

tissue, instead of being controlled, as normally, by nerve impulses arising from the marginal sense organs (fig. 6). Sixth, specimens with insulated halves in one-half of which muscular contraction was maintained by means of a circuit wave of contraction while the pulsation of the other half was under the normal control of its sense organs (fig. 7).

EXPERIMENTS WITH ENTIRE DISKS

When the rate of regeneration of a series of active and inactive entire disks is compared it is found that in about 75 per cent of all the experiments the regeneration is most rapid in the active

specimens. In the remaining disks the amount regenerated at any given time is, in about 10 per cent of the pairs, found to be equal within the limits of accuracy of measurement, while in about 15 per cent of the pairs of disks regeneration was greatest in the inactive specimens.

The results of many different kinds of experiments upon Cassiopea have shown that there are wide variations in the sensitivity and metabolic activity in this animal. It therefore seems evi-

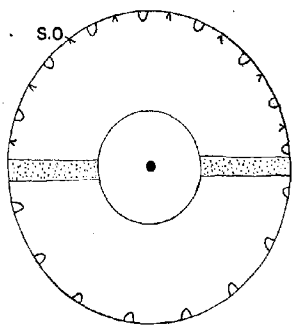


Fig. 3 To illustrate the type of operation (Stockard's) used in experiments of types 3 and 4. The sense organs were removed from one-half of the disk, and an equal amount of tissue from the margin between the sense organs of the other half of the disk. The two halves were then insulated by scraping off two diametrically opposite strips of subumbrella ectoderm. The insulating strips are shown stippled in the figure.

dent that the conflicting results obtained from specimens subjected to this type of operation are to be explained as individual variations in physiological activity.

More dependable results may be expected from specimens prepared according to Stockard's method (fig. 3) where individual variations in physiological activity are eliminated.

After this operation the inactive half of each specimen is moved about by the pulsation of the active half so that there can be little difference in the degree of aeration of any two parts of the disk. In all experiments of this type (2) where large numbers

of specimens were used two difficulties were met in making the measurements. Frequently the disk became folded backward at the point where the subumbrella ectoderm was removed sometimes even bringing the exumbrella surfaces in contact. While this seemed in no way to interfere with regeneration it frequently made accurate measurements impossible unless the specimen was first narcotized, as any attempt to unfold the active disk usually resulted in tearing the delicate regenerating tissue. As this procedure involved the expenditure of so much time all badly folded specimens were discarded. If the folding took place some days after the start of any series of experiments the specimen was discarded and the figures for the earlier stages retained in the record.

The other most common source of difficulty in making the measurements arose on account of the tendency of the edge of the regenerating tissue to fuse with the edge of the old cut surface or with a more proximal part of the sheet of regenerating tissue. Whenever the edge of the thin sheet of new tissue became folded back sufficiently to touch any of the more proximal tissues fusion took place so that a tube would be formed from the new tissue. When the folding involved only a small area separation could be easily accomplished, but if a considerable portion of the regenerating sheet was involved the specimen was rendered useless for further study.

The results of two typical experiments are shown in table 1. The measurements are in millimeters. The upper figure for each date shows the width of the sheet of tissue regenerated from the active half disk, the lower figure the width of that regenerated from the passive half disk. When the sheet of new tissue had entirely closed over the cavity in the center of the disk the point of closure remained recognizable for at least a day so that the measurements could readily be made for those disks that had become closed since the time of the last measurements. By the end of twenty-four hours after the new sheet of tissue was completed the point of closure would be shifted until it came to lie in the center of the disk.

TABLE 1

Record of experiment 4a started September 30, 1913. 40 disks each with all sense organs removed from one side and with the halves insulated by removal of 2 strips of subumbrella ectoderm

NUMBER OF SPECIMEN		DAYS AFTER OPERATION				
		1	2	3	4	5
1	Half with S. O.	3.25	4.75	9.50 C		
	Half without S. O.	2.00	2.50	6.00		
2	Half with S. O.	2.50	4.00	5.00	9.00 C	
	Half without S. O.	1.50	3.00	3.75	6.25	
3	Half with S. O.	2.25	3.00	5.50	8.25 C	
	Half without S. O.	1.75	2.00	3.25	5.00	
4	Half with S. O.	2.75	4.00	5.75	14.75 C	
	Half without S. O.	1.75	3.00	4.50	10.50	
5	Half with S. O.	2.00	3.75	5.50	7.25 C	9.00
	Half without S. O.	1.50	3.00	4.75	6.00	7.50
6	Half with S. O.	1.50	3.00	3.75	5.00	6.75 C
	Half without S. O.	0.80	2.25	2.75	3.25 C	4.50
7	Half with S. O.	3.75	5.00	5.75	7.50	
	Half without S. O.	1.75	2.00	3.00	5.00	
8	Half with S. O.	3.50	4.00	4.00	9.50 C	
	Half without S. O.	2.50	3.00	3.00	7.00	
9	Half with S. O.	2.75	3.75	5.00	5.25	6.00 C
	Half without S. O.	2.25	3.00	3.85	4.00	4.30
10	Half with S. O.	3.25	5.50	8.25	9.50 C	
	Half without S. O.	2.50	4.00	6.50	4.25	
11	Half with S. O.	3.00	4.00	6 00	8.00 C	
	Half without S. O.	2.15	3.00	4.00	6.25	
12	Half with S. O.	2.50	5.00	6.50	7.50	9.00 C
	Half without S. O.	1.75	3.75	4.50	5.25	6.50
13	Half with S. O.	1.50	4.00	4.25	6.00	8.75 C
	Half without S. O.	1.00	3.75	4.00	5.00	7.00
14	Half with S. O.	4.00	5.00	6.80	7.00 C	
	Half without S. O.	3.00	4.00	5.25	5.25	
15	Half with S. O.		3.00	4.50	7.00	10.00
	Half without S. O.		2.00	3.50	5.50	7.00
16	Half with S. O.	2.50	3.75	4.75	6.00	10.50 C
	Half without S. O.	1.50	2.50	3.25	4.15	8.00
17	Half with S. O.	2.75	3.75	6.25	7.75 C	
	Half without S. O.	2.50	3.00	5.00	6.00	
18	Half with S. O.	3.00	4.00	5.50	8.00 C	
	Half without S. O.	2.00	3.00	3.75	5.75	
19	Half with S. O.	2.75	3.50	4.25	6.00 C	
	Half without S. O.	2.75	3.00	3.50	5.00	
20	Half with S. O.	2.75	4.00	6.00	9.00	14.00 C
	Half without S. O.	2.00	2.50	3.75	7.00	9.00

TABLE 1—Continued

NUMBER OF SPECIMEN		DAYS AFTER OPERATION				
		1	2	3	4	5
21	Half with S. O.	2.50	3.50	4.50	7.00 C	
	Half without S. O.	1.50	2.75	3.50	5.50	
22	Half with S. O.	2.75	3.50	Grown	together	
	Half without S. O.	1.50	2.50			
23	Half with S. O.	3.00	4.25	5.75	9.00	
	Half without S. O.	2.00	3.00	4.25	8.25	
24	Half with S. O.	2.00	4.50	5.50 C		
	Half without S. O.	1.25	2.75	3.50		
25	Half with S. O.	2.25	4.75	8.00 C		
	Half without S. O.	1.50	3.15	5.50		
26	Half with S. O.	2.50	3.50	5.50	8.25 C	
	Half without S. O.	1.50	2.25	3.75	6.50	
27	Half with S. O.	2.00	4.50	6.75 C		
	Half without S. O.	1.50	3.75	5.00		
28	Half with S. O.	2.25	4.00	6.00 C		
	Half without S. O.	1.50	3.00	4.50		
29	Half with S. O.	2.50	3.75	4.50	Badly wrinkled	
	Half without S. O.	2.00	3.00	3.50		
30	Half with S. O.	3.00	3.55	3.75	4.75	7.00 C
	Half without S. O.	1.20	2.00	2.50	3.25	5.50
31	Half with S. O.	2.00	3.00	5.00	7.00 C	
	Half without S. O.	1.00	2.25	3.50	5.00	
32	Half with S. O.	2.00	3.00	6.50	10.75 C	
	Half without S. O.	1.25	2.00	4.00	7.50	
33	Half with S. O.	1.75	2.50	4.50	6.00 C	
	Half without S. O.	0.50	1.00	2.50	4.00	
34	Half with S. O.	2.00	4.00	6.00 C		
	Half without S. O.	1.50	3.00	4.00		
35	Half with S. O.	3.00	Too badly wrinkled to measure new tissue			
	Half without S. O.	2.00				
36	Half with S. O.	3.75	8.25 C			
	Half without S. O.	2.00	5.00			
37	Half with S. O.	2.75	4.00	7.00 C		
	Half without S. O.	1.50	3.00	5.50		
38	Half with S. O.	1.50	2.25	Badly folded		
	Half without S. O.	1.00	1.75			
39	Half with S. O.	2.00	5.25	7.50 C		
	Half without S. O.	1.25	3.00	5.25		
40	Half with S. O.	4.25	6.00 C			
	Half without S. O.	3.00	4.75			

TABLE 1—Continued

Mean of all observations

NUMBER OF SPECIMENS	DAYS AFTER OPERATION				
	1	2	3	4	5
Half with S. O.	2.65	4.05	5.75	7.63	9.00
Half without S. O.	1.73	2.87	4.06	5.57	6.36

Explanation of table: The letter C after the measurement for any day indicates that the central activity had been filled by the regenerated tissue.

The results from the forty disks recorded in table I are shown in figure 4 in which the divisions along the abscissa show the

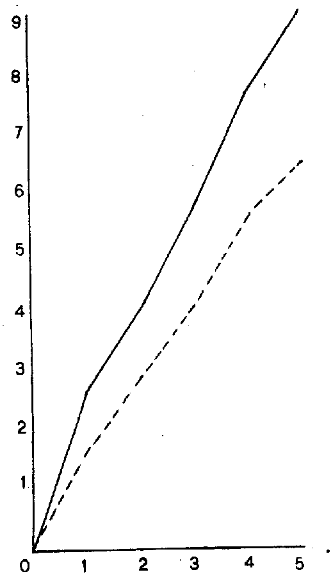


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Fig. 4 Showing the relative rates of regeneration of the halves of 40 disks The upper (solid) curve is for the half disk with sense organs, the lower (broken) curve is for the half disk without sense organs. The divisions along the ordinate represent the amount of regeneration in millimeters. Those along the abcissa the time of regeneration in days.

number of days during which the regeneration has been going on, those along the ordinate the amount of regeneration in millimeters. The record for each specimen is carried to the time of closure of the open circle in the center of the disk by the sheet of regenerated tissue.

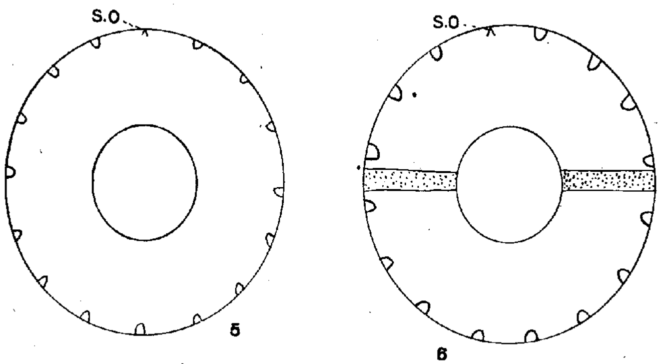
From the start of regeneration the new tissue produced from the side with its sense organs intact is shown to be more rapid. In the early stage of regeneration this difference is more striking upon a cursory examination than in the later stages although the actual difference on the rate of growth of new tissue changes only slightly during the entire period of regeneration. The proportion between the amounts of new tissue formed each day, taking the amount regenerated from the side without sense organs as the unit, was respectively: First day 1 : 1.53; second day 1 : 1.44; third day 1 : 1.41; fourth day 1 : 1.38; fifth day 1 : 1.39.

The regeneration from the half without sense organs is more regular as is shown by the fact that for the mean of each day's observation the probable error is less for that half than for the one upon which the sense organs remain. This result would be expected to follow from the fact that the inactive side was relieved from the influence of the marginal sense organs which would introduce many stimuli of varying intensity all of which would have either a retarding or accelerating influence upon the processes of regeneration.

When the rates of regeneration of certain disks in table 1 are compared with one another the cause of the uncertainty of the results obtained in experiments with entire disks is clearly shown. The closure of the open circle in disk 36, table 11, was complete in two days while five days were necessary to complete the closure in specimen 30, table 1. Had the latter been the active disk, and the former the inactive disk, of a pair compared in an experiment with entire disks the conclusion that an inactive disk sometimes degenerates more rapidly than an active one could not have been avoided.

EXPERIMENTS OF TYPE 1A AND 2A

Mayer ('06) has pointed out the fact that in a disk of Cassiopea a single marginal sense organ will control pulsation just as effectively as will the sixteen normally present. His studies have shown besides that the most rapidly discharging sense organ really determines the rate of pulsation in a normal medusa. The experiments of type 1a and 2a were undertaken to determine whether or not a single marginal sense organ would show the same influence on the rate of regeneration as upon pulsation.



Figs. 5 and 6 To show the operation used in experiments of types 1a and 2a. In figure 5 one sense organ only is left but its influence can be transmitted throughout the disk. In figure 6 a single sense organ is left while the two halves of the disk are insulated so that the influence of the sense organ is confined to one-half.

The results obtained from the experiments of type 1a (fig. 4) were, as in all the experiments with entire disks, inconstant. Usually disks with their sense organs intact regenerated most rapidly. In a relatively small number of pairs the rate was the same for each member of a pair and in two instances the disk without sense organs regenerated faster than the one upon which the sense organs remained. Here again as in experiments of type 1 the individual variation in physiological activity of the different disks affords an explanation of the irregularity of the results obtained.

For the experiments of type 2a (fig. 5) where the disks consisted of an active and an inactive half there was shown a constancy of results. The active half invariably showed the first evidences of regeneration and maintained a higher rate throughout the entire period until the cavity in the center of the disk had been closed. So far as is shown by a comparison of the amount of tissue regenerated in a given time by disks under the conditions in experiments of type 2 and type 2a there was no noticeable difference in the rate of regeneration when only a single sense organ is left on the active half disk or when the full number of sense organs was present.

The results of these studies on the influence of the sense organs on the rate of regeneration confirm Mayer's observations that a single sense organ is sufficient to supply the nervous influences that control the normal activities of a medusa.

EXPERIMENTS OF TYPE 3 (ANESTHETICS)

In the experiments of type 2 and 2a it was shown that the active half of a medusa disk prepared as shown in figures 3 and 5 has a higher rate of regeneration than does the inactive half of the same specimen. While this point is clearly shown in all the experiments of the two types just mentioned, the results obtained by this method throw no light upon the nature of the control exercised by the marginal sense organs, as to whether it is exerted through the higher metabolism brought about by muscular activity, or through some other less apparent metabolic process under the control of the sense organs.

Two other types of experiments were undertaken to ascertain the nature of the nervous control. In the first set of experiments (type 3) disks prepared with insulated active and inactive halves (fig. 3) were allowed to regenerate in sea water to which fifteen per cent by volume of 0.6 M $MgSO_4$ had been added. In this solution the disks will live for an indefinite time and will for several hours retain the capacity to regain their normal activity within a few moments after being returned to fresh sea water. Mayer (op. cit) has shown that in a weak $MgSO_4$ solution in sea water the effect of the magnesium is for a time confined almost

entirely to the muscular tissues, while the nervous network is still capable of transmitting the impulse necessary for pulsation over an area submerged in the magnesium solution where no contraction of the muscles could be observed. When kept in the magnesium sea water for a prolonged period the sense organs become incapable of giving rise to the stimulus necessary for normal pulsation long before the nervous network loses its capacity for transmitting such a stimulus, so that a ring cut from a medusa disk and activated by a circuit wave of contraction will show by an indicator strip in sea water (Mayer, l.c., page 122) the transmission of the nervous impulse for a considerable time after a ring that retains its sense organs is no longer able to activate its indicator strip.

When medusa disks prepared with insulted active and inactive halves are put into the magnesium sea water they lose their power of muscular movement within a few moments. Usually all of the disks float on the surface of the new solution for from twenty minutes to a half hour before they become adjusted to the abnormally dense medium. At the end of this period they settle to the bottom of the jar and remain completely relaxed throughout the experiment.

During the first twelve hours of an experiment, or as soon as the newly regenerated tissue became recognizable regeneration is more rapid from the side on which the sense organs are present. From that time on the regeneration is (within the limits of error of the measurements) about equal from the two halves. The rate of regeneration of the half disks with sense organs falls to equal that of the half without sense organs. For both halves the rate was noticeably lower than that of the inactive half of a disk in normal sea water. The lack of proper aëration commonly brought about through the pulsation of the active half disk may account in part for the lower rate of regeneration but there is unquestionably some more fundamental disturbance in the metabolic activity caused by the presence of the excess of Mg ions in the fluid.

Experiments with chloroform and with KCN dissolved in sea water did not give satisfactory results. In both these solutions the tissues of the medusa underwent rapid disintegration if the

TABLE 2

Record of experiments 12 and 12a. 40 disks each with insulated halves, one with sense organs, the other without. Mg. sea water

NUMBER OF SPECIMEN		DAYS OF REGENERATION 1	2	3	4	5	6	7	8	9
1	Half with S. O.	2.00	2.50	3.25	3.75	4.50	5.25	6.00	6.60	6.40
	Half with out S. O.	0.80	1.40	2.00	2.75	3.40	4.20	4.80	5.70	6.55
2	Half with S. O.	1.75	2.30	2.90	3.75	4.25	5.00	6.00	6.50	7.25
	Half without S. O.	0.90	1.50	1.75	2.50	3.25	4.00	4.75	5.25	6.00
3	Half with S. O.	1.00	1.50	2.75	3.75	4.50	5.00	5.75	6.50	7.00
	Half without S. O.	0.50	0.75	1.25	2.00	3.00	3.75	4.50	5.25	6.25
4	Half with S. O.	1.50	2.25	3.00	3.50	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	1.00	1.50	2.00	2.75	3.25	3.75	4.50	5.25	6.00
5	Half with S. O.	1.75	2.25	3.00	3.75	4.50	5.25	5.75	6.25	7.00
	Half without S. O.	1.00	1.50	2.25	2.00	3.50	4.00	4.50	5.00	5.75
6	Half with S. O.	2.00	2.75	3.50	4.25	5.25	5.75	6.25	7.00	C
	Half without S. O.	1.25	1.75	2.50	3.00	3.75	4.25	4.75	5.75	
7	Half with S. O.	1.00	2.00	3.00	3.75	4.75	5.50	6.25	7.00	7.50
	Half without S. O.	0.75	1.25	2.00	2.50	3.25	4.00	4.50	5.25	5.75
8	Half with S. O.	1.25	1.50	2.25	3.00	4.00	4.75	5.50	6.25	7.00
	Half without S. O.	0.75	1.00	1.25	1.75	3.50	3.25	3.75	4.50	5.25
9	Half with S. O.	2.25	2.75	3.50	4.00	4.75	5.50	6.25	7.25	C
	Half without S. O.	1.00	1.50	2.25	2.75	3.25	4.00	4.50	5.25	
10	Half with S. O.	1.50	2.25	3.00	3.50	4.25	5.00	5.50	6.25	7.25
	Half without S. O.	0.50	1.00	1.75	2.25	3.00	3.75	4.50	5.25	6.00
11	Half with S. O.	1.00	1.75	2.50	3.50	4.25	5.00	5.75	6.50	7.25
	Half without S. O.	0.50	1.00	1.75	2.50	3.25	4.00	4.50	5.25	6.00
12	Half with S. O.	2.50	3.25	4.00	4.75	5.50	6.25	7.00	7.75	C
	Half without S. O.	1.25	1.75	2.25	2.75	3.25	4.00	4.50	5.25	
13	Half with S. O.	1.75	2.25	3.00	3.75	4.50	5.25	5.75	6.50	7.25
	Half without S. O.	0.50	1.25	1.75	2.50	3.25	4.00	4.75	5.50	6.00
14	Half with S. O.	0.75	1.50	2.25	2.75	3.75	4.50	5.25	6.25	7.25
	Half without S. O.	0.25	0.75	1.50	2.25	3.00	3.75	4.50	5.25	6.25
15	Half with S. O.	1.50	2.00	2.75	3.75	4.50	5.25	6.00	6.75	7.25
	Half without S. O.	0.50	1.25	1.75	2.50	3.25	4.00	4.75	5.25	6.00
16	Half with S. O.	2.00	2.75	3.25	4.00	4.50	5.25	6.00	6.50	7.25
	Half without S. O.	0.75	1.25	1.75	2.50	3.25	4.00	4.75	5.25	6.00
17	Half with S. O.	1.75	2.50	3.25	4.00	4.75	5.25	6.00	6.75	7.50
	Half without S. O.	0.50	1.25	2.00	2.50	3.25	4.00	4.75	5.25	6.00
18	Half with S. O.	2.25	3.00	3.75	4.50	5.00	6.00	7.00	C	
	Half without S. O.	1.00	1.50	2.25	2.75	3.50	4.25	5.25		
19	Half with S. O.	2.75	3.25	4.50	5.25	6.00	6.75	7.50	C	
	Half without S. O.	1.50	2.00	2.50	3.25	3.75	4.25	5.00		
20	Half with S. O.	1.50	2.25	3.00	3.75	4.50	5.50	6.25	7.00	7.75
	Half without S. O.	0.75	1.50	2.00	2.50	3.25	4.00	4.75	5.25	6.00
21	Half with S. O.	3.00	3.50	4.25	4.75	5.25	6.00	6.75	7.50	C
	Half without S. O.	1.50	2.00	2.50	3.25	3.75	4.50	5.25	6.00	

TABLE 2—Continued

NUMBER OF SPECIMEN		DAYS OF REGENERATION								
		1	2	3	4	5	6	7	8	9
22	Half with S. O.......	2.25	2.75	3.25	3.75	4.50	5.25	6.00	6.50	7.25
	Half without S. O....	1.00	1.50	2.00	2.50	3.25	4.00	4.75	5.50	6.25
23	Half with S. O.......	1.50	2.25	3.00	3.75	4.25	5.00	5.75	6.50	7.25
	Half without S. O....	0.75	1.25	2.00	2.75	3.50	4.25	5.00	5.75	6.00
24	Half with S. O.......	2.00	2.50	3.25	3.75	4.50	5.00	5.75	6.25	7.00
	Half without S. O....	0.75	1.50	2.25	1.75	3.25	3.75	4.50	5.00	5.75
25	Half with S. O.......	1.25	2.00	2.75	3.50	4.75	5.25	6.00	6.75	7.50
	Half without S. O....	0.50	1.00	1.50	2.25	2.00	3.50	4.25	5.00	6.00
26	Half with S. O.......	1.75	2.25	3.00	4.00	4.75	5.25	6.00	7.00	8.00
	Half without S. O....	0.50	1.00	1.50	2.50	3.25	3.75	4.50	5.25	6.25
27	Half with S. O.......	2.50	3.25	4.00	4.75	5.50	6.50	7.50	8.25	C
	Half without S. O....	1.00	1.75	2.50	3.25	4.00	4.75	5.50	6.25	
28	Half with S. O.......	2.25	3.00	3.50	4.00	4.75	5.50	6.25	7.00	7.75
	Half without S. O....	1.00	1.75	2.25	3.00	3.50	4.00	4.75	5.25	6.00
29	Half with S. O.......	1.75	2.50	2.00	3.75	4.25	5.00	5.75	6.50	7.25
	Half without S. O....	1.00	1.75	2.25	2.75	3.50	4.25	5.00	5.75	6.50
30	Half with S. O.......	2.75	3.25	4.00	5.00	6.00	6.75	7.50	C	
	Half without S. O....	1.75	1.50	2.25	3.00	3.75	4.00	5.00		
31	Half with S. O.......	2.25	3.00	3.75	4.50	5.25	6.00	6.75	7.50	C
	Half without S. O....	1.00	1.50	2.25	3.00	3.75	4.50	5.25	6.00	
32	Half with S. O.......	1.50	2.50	3.25	3.75	4.50	5.25	6.00	6.75	7.50
	Half without S. O....	0.50	1.25	2.00	2.75	3.50	4.25	5.00	5.75	6.25
33	Half with S. O.......	2.00	2.75	3.25	4.00	4.50	5.25	6.00	6.75	7.50
	Half without S. O....	0.75	1.50	2.00	2.75	3.25	4.00	4.75	5.50	6.25
34	Half with S. O.......	1.00	1.75	2.50	3.25	4.00	5.00	5.75	6.50	7.25
	Half without S. O....	0.25	1.00	1.75	2.00	2.75	3.50	4.25	5.00	5.75
35	Half with S. O.......	1.75	2.50	3.25	3.75	4.50	5.26	6.00	6.75	7.50
	Half without S. O....	1.00	1.50	2.25	2.75	3.50	4.25	4.75	5.50	6.25
36	Half with S. O.......	2.25	3.00	3.50	4.00	4.50	5.25	6.00	6.50	7.25
	Half without S. O....	1.00	1.75	2.50	3.25	4.00	4.75	5.50	6.00	6.75
37	Half with S. O.......	1.50	2.25	3.00	3.75	4.50	5.25	6.00	6.75	7.75
	Half without S. O....	0.75	1.50	2.25	2.75	3.50	4.25	5.00	5.75	6.50
38	Half with S. O.......	2.75	3.50	4.25	5.00	5.75	6.50	7.25	8.00	C
	Half without S. O....	1.00	1.75	2.50	3.25	4.00	4.75	5.50	6.00	
39	Half with S. O.......	2.25	3.00	3.50	4.25	5.00	5.50	6.00	6.75	7.50
	Half without S. O....	0.75	1.50	2.25	3.00	3.75	4.50	5.00	5.75	6.50
40	Half with S. O.......	1.50	2.25	3.00	3.50	4.25	5.00	5.75	6.50	7.25
	Half without S. O....	0.50	1.25	2.75	2.75	3.50	4.25	5.00	5.75	6.50

Mean of all observations

	Half with S. O.......	2.00	2.35	2.92	3.55	4.57	5.17	5.94	6.47	7.40
	Half without S. O....	0.85	1.43	2.00	2.85	3.45	4.23	4.87	5.74	6.47

amount of the reagent present was sufficient to bring about any noticeable effect upon the activity of the sense organs.

When medusae are treated with a weak solution of oxalic acid in magnesium-free artificial sea water it is possible to destroy the activity of the sense organs for a considerable time without

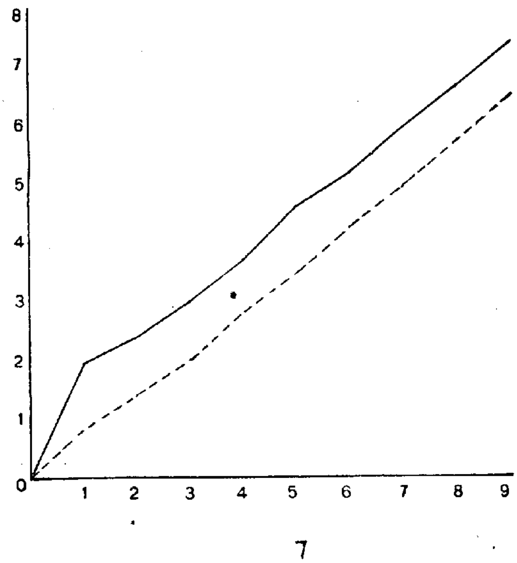


Fig. 7 Curves to show the rate of regeneration of the halves of 40 disks, one-half with and one-half without sense organs regenerating in sea water to which had been added 15 parts of 0.6 M $MgSO_4$. The upper (solid) line shows the regeneration of the half disk with sense organs. The lower (broken) line shows regeneration of the half disk without sense organs. The divisions along the ordinate represent the amount of regeneration in millimeters. Those along the abscissa the time of regeneration in days.

seriously injuring the other tissues. In all my experiments, however, recovery of functional activity by the sense organs took place within twenty-four hours if the oxalic acid solution was of such a strength that the ectodermal tissues were not injured. In such experiments there was at first an equal rate of regenera-

tion for each of the halves of a disk until the sense organs regained their functional activity after which the half disk with sense organs regenerated most rapidly, as in experiments of type 2.

The results obtained in experiments with Mg solutions show that there is an influence of the sense organs on the rate of regeneration which is apparently exercised for a considerable time after muscular activity has been suppressed. It was impossible, however, by this method to differentiate with any certainty between the two effects, since there is no visual means of

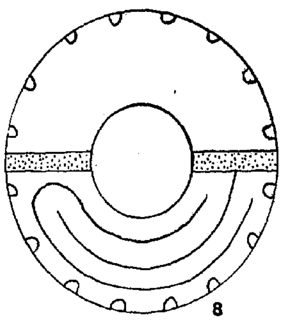


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Fig. 8 To show the character of the operation in experiments of type 5. All sense organs were removed from the disk. The two halves insulated by removal of appropriate strips of subumbrella ectoderm. One half was kept in pulsation by means of a circuit wave of contraction running through an endless labyrinth of subumbrella tissue.

ascertaining at what time the sense organs lose their power of sending out the stimulus necessary for normal contraction.

Since, as was shown by Mayer (op. cit), it is possible to maintain a circuit wave of contraction in the muscles of a half disk without sense organs by making a series of cuts by which an endless labyrinth of subumbrella tissue is formed (fig. 8), the part played by muscular activity and that by the stimuli from the sense organs can be directly compared.

In experiments of type 5 (fig. 8) all the sense organs were removed from the medusa disks, the halves insulated, and a circuit wave of contraction started by an induction shock in the laby-

rinth cut in the muscle tissues of one of its halves. Once established the contraction wave would be maintained throughout the course of an experiment unless interrupted by an unusually strong stimulus through some accident in handling. When interrupted in this way the circuit wave could be established again by renewed electrical stimulation. The amplitude of the contraction wave becomes gradually reduced as time goes on, but there is little variation in its rate. When the rates of regeneration of the halves of any disk prepared in this manner are compared it is found that the half in which the circuit wave is maintained regenerates slightly faster than the inactive one. This difference in rate is, however, very much less than between the halves of a disk from one-half of which the sense organs have been removed (compare figs. 4 and 9) although the activated disk pulsates on the average more than three times as fast as one under the control of the sense organs. The amount of activity and metabolism in the muscles if they have any noticeable influence on the rate of regeneration ought to produce a clearly demonstrable result, but as shown by the data in table 3 and figure 9 the difference is relatively small. From the point of view of the chemical nature of metabolism (including regeneration) the difference in temperature might conceivably be sufficient to account for the observed difference in rate of regeneration. The half disks in which the circuit wave is maintained show a greater regularity in the rate of regeneration than do the active disks in experiments of type 2 (see table 1).

The records for 40 disks used in an experiment of this type are shown in table 3 and figure 9. A further demonstration of the influence of the sense organs on the rate of regeneration is furnished in another series of experiments,—type 6, figure 10—in which the two insulated halves of a disk are compared, one of which is contracting normally under the influence of its sense organs while all the sense organs are removed from the other half and a circuit wave of contraction maintained in a labyrinth of its subumbrella muscles. In a disk prepared in this manner the rate of pulsation will be on the average more than three times as great in the activated half disk as in that contracting under the

TABLE 3

Record of 40 specimens from which all of the sense organs had been removed. and the subumbrel a muscles of one half activated by a circuit wave of contraction (fig. 8)

NUMBER OF SPECIMEN		DAYS OF REGENERATION					
		1	2	3	4	5	6
1	Activated half......	2.00	3.25	4.50	6.25	7.25	8.00
	Half without S. O...	1.75	2.75	4.00	5.50	6.50	7.25
2	Activated half......	2.50	3.25	4.75	6.50	8.00	C
	Half without S. O...	2.00	3.00	4.25	6.00	7.50	
3	Activated half......	1.75	3.00	4.50	6.00	7.00	8.25
	Half without S. O...	1.50	2.25	3.25	5.25	6.25	7.00
4	Activated half......	1.50	2.75	4.00	6.00	7.25	8.25
	Half without S. O...	1.25	2.50	3.50	5.25	6.00	7.00
5	Activated half......	2.25	3.25	4.50	6.25	7.00	8.25
	Half without S. O...	1.75	2.75	4.00	5.50	6.25	7.25
6	Activated half......	2.00	3.25	4.75	6.00	7.50	8.50
	Half without S. O...	1.75	2.75	4.00	5.50	6.50	7.25
7	Activated half......	2.75	4.00	5.00	6.50	8.00	C
	Half without S. O...	2.25	3.25	4.25	5.50	7.00	
8	Activated half......	3.00	4.00	5.25	6.50	8.00	C
	Half without S. O...	2.50	3.00	4.50	5.75	7.25	
9	Activated half......	1.00	2.25	3.50	5.25	6.50	8.00
	Half without S. O...	0.75	1.75	3.00	3.75	5.75	7.25
10	Activated half......	1.75	3.00	4.25	6.00	7.50	8.25
	Half without S. O...	1.50	2.50	3.50	5.00	6.25	7.00
11	Activated half......	2.50	3.25	4.50	6.25	7.25	8.00
	Half without S. O...	2.25	2.75	4.00	3.75	6.75	7.50
12	Activated half......	3.50	4.25	5.25	6.75	8.25	C
	Half without S. O...	3.25	3.75	4.75	6.00	7.50	
13	Activated half......	1.25	2.50	4.25	6.00	7.50	8.50
	Half without S. O...	1.00	2.25	4.00	5.50	6.75	7.25
14	Activated half......	2.00	3.25	4.50	6.25	7.25	8.25
	Half without S. O...	1.75	2.75	4.00	5.75	6.50	7.25
15	Activated half......	2.25	3.50	4.50	6.50	8.00	C
	Half without S. O...	2.00	3.00	4.00	6.00	7.25	
16	Activated half......	1.00	2.50	4.25	6.00	7.25	8.25
	Half without S. O...	0.75	2.25	4.00	5.00	6.25	7.00
17	Activated half......	1.75	3.00	4.25	6.00	7.25	8.00
	Half without S. O...	1.50	2.50	3.75	5.50	6.75	7.50
18	Activated half......	2.50	3.50	4.50	6.50	8.50	C
	Half without S. O...	2.25	3.00	4.00	6.00	7.75	
19	Activated half......	1.25	2.75	4.00	6.00	7.00	7.75
	Half without S. O...	1.00	2.50	3.75	5.75	6.75	7.50
20	Activated half......	2.00	3.25	4.50	6.00	7.25	8.00
	Half without S. O...	1.75	2.75	4.00	5.75	6.50	7.25

TABLE 3—Continued

NUMBER OF SPECIMEN		DAYS OF REGENERATION					
		1	2	3	4	5	6
21	Activated half......	1.75	3.00	4.60	6.25	7.25	8.00
	Half without S. O...	1.50	2.75	4.00	5.50	6.50	7.25
22	Activated half......	2.50	3.25	4.50	6.00	7.00	8.25
	Half without S. O...	2.00	2.75	4.00	5.50	6.25	7.25
23	Activated half......	2.00	3.00	4.50	6.00	7.00	8.00
	Half without S. O...	1.75	2.75	4.00	5.25	6.25	7.25
24	Activated half......	3.00	3.75	5.00	6.25	8.00	C
	Half without S. O...	2.50	3.25	4.25	5.50	7.25	
25	Activated half......	1.50	2.50	4.25	5.75	7.00	8.00
	Half without S. O...	1.25	2.25	4.00	5.50	6.50	7.50
26	Activated half......	1.00	2.50	4.50	6.25	7.00	8.00
	Half without S. O...	0.75	2.25	4.00	5.50	6.25	7.25
27	Activated half......	2.00	3.25	4.50	6.50	7.25	8.00
	Half without S. O...	1.75	3.00	3.75	5.75	6.50	7.25
28	Activated half......	2.25	3.25	4.50	6.25	7.00	8.00
	Half without S. O...	1.75	2.75	4.00	5.50	6.25	7.25
29	Activated half......	2.00	3.00	4.75	6.25	7.25	8.00
	Half without S. O...	1.75	2.75	4.00	5.50	6.25	7.00
30	Activated half......	1.50	2.50	4.00	6.00	7.00	8.25
	Half without S. O...	1.25	2.25	3.75	5.50	6.50	7.25
31	Activated half......	2.00	3.00	4.50	6.00	7.25	8.00
	Half without S. O...	1.50	2.50	4.00	5.25	6.25	7.00
32	Activated half......	2.75	3.50	4.75	6.75	7.50	8.50
	Half without S. O...	2.25	3.00	4.00	5.75	6.75	7.25
33	Activated half......	4.00	5.75	7.75	8.75	C	
	Half without S. O...	3.50	4.75	7.00	7.75		
34	Activated half......	2.00	3.50	5.00	6.50	8.00	C
	Half without S. O...	1.75	3.00	4.25	5.75	7.50	
35	Activated half......	1.25	2.50	4.00	6.00	7.50	8.50
	Half without S. O...	1.00	2.25	3.75	5.75	6.50	7.25
36	Activated half......	1.00	2.50	4.25	6.00	7.25	8.00
	Half without S. O...	0.75	2.00	3.75	5.50	6.75	7.50
37	Activated half......	1.50	3.00	4.75	6.25	7.25	8.00
	Half without S. O...	1.25	2.75	4.25	5.50	6.50	7.25
38	Activated half......	2.00	3.00	4.25	6.00	7.25	8.25
	Half without S. O...	1.75	2.75	4.00	5.50	6.50	7.25
39	Activated half......	1.00	2.25	4.00	6.00	7.00	8.25
	Half without S. O...	0.75	2.00	3.75	5.25	6.25	7.50
40	Activated half......	2.00	3.00	4.50	6.50	7.50	8.50
	Half without S. O...	1.75	2.50	4.00	5.50	6.25	7.00

Mean of all observations

	1	2	3	4	5	6
Activated half......	2.00	3.27	4.67	6.15	7.23	8.12
Half without S. O...	1.73	2.87	4.06	5.57	6.36	7.27

influence of its sense organs. The half disk with its sense organs, although pulsating much more slowly than its activated mate shows a considerably higher rate of regeneration than the latter.

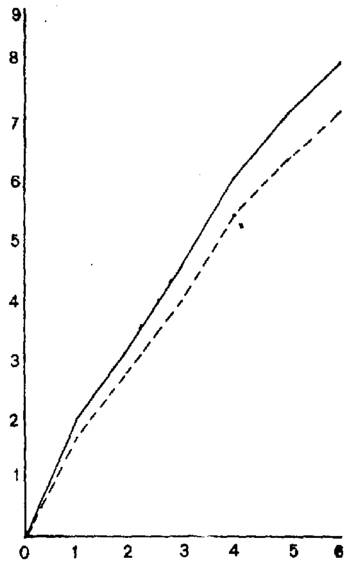

Fig. 9 Curves to show the comparative rates of regeneration between the halves of 40 disks from which all of the sense organs have been removed, while the subumbrella muscles of one-half of each disk are activated by a circuit wave of contraction. The upper (solid) line shows the regeneration of the activated half disks, while the lower (broken) line shows the regeneration of the half disks without sense organs or activation. The divisions along the ordinate represent the amount of regeneration in millimeters. Those along the abscissa the time of regeneration in days.

That the influence of the sense organs upon the rate of regeneration differs in no essential respect from that exercised upon the general metabolic activity of the entire disk is shown by comparing the amounts of CO_2 produced in a given time by half

TABLE 4

Record of 40 specimens, one half of each one pulsating under the control of its sense organs; while a circuit wave of contraction was maintained in the sub-umbrella muscles of the other half (fig. 10).

NUMBER OF SPECIMEN		DAYS OF REGENERATION 1	2	3	4	5
1	Half with S. O.	3.00	4.25	5.75	7.50	9.00
	Activated half	2.25	3.25	4.50	6.00	7.50
2	Half with S. O.	1.75	3.50	5.50	7.25	9.00
	Activated half	1.50	3.25	5.25	7.00	8.00
3	Half with S. O.	4.00	5.50	6.75	8.25	C
	Activated half	3.50	4.75	5.50	7.50	
4	Half with S. O.	2.50	4.25	5.50	7.50	9.50
	Activated half	2.00	3.75	4.75	6.75	8.00
5	Half with S. O.	2.00	4.00	5.50	7.50	9.00
	Activated half	1.50	3.25	4.50	6.00	7.50
6	Half with S. O.	2.75	4.25	5.75	7.75	9.25
	Activated half	2.00	3.50	4.75	6.50	7.50
7	Half with S. O.	2.50	4.00	5.50	7.25	8.75
	Activated half	2.00	3.25	4.75	6.25	7.75
8	Half with S. O.	2.25	4.25	5.75	7.50	9.00
	Activated half	1.75	3.00	4.25	6.00	7.50
9	Half with S. O.	3.00	4.50	5.75	7.75	9.25
	Activated half	2.50	3.75	5.00	6.50	7.50
10	Half with S. O.	1.50	3.00	5.00	7.00	9.00
	Activated half	1.25	2.75	4.50	6.25	8.00
11	Half with S. O.	2.25	4.00	5.50	7.00	9.00
	Activated half	1.75	3.25	4.75	6.25	7.50
12	Half with S. O.	3.00	4.75	5.50	7.75	9.25
	Activated half	2.25	3.25	4.50	6.00	7.25
13	Half with S. O.	2.50	4.00	5.75	7.25	9.00
	Activated half	2.00	3.50	4.75	6.25	7.25
14	Half with S. O.	2.75	4.25	5.75	7.75	9.25
	Activated half	2.25	3.50	4.50	6.50	7.75
15	Half with S. O.	3.00	4.25	5.75	8.00	9.25
	Activated half	2.25	3.25	4.75	6.25	7.50
16	Half with S. O.	4.00	6.00	7.75	9.50	C
	Activated half	3.25	5.25	7.00	8.25	
17	Half with S. O.	2.50	4.25	5.50	7.25	9.00
	Activated half	2.00	3.25	4.75	6.50	7.75
18	Half with S. O.	2.00	4.00	5.75	7.25	9.25
	Activated half	1.50	3.50	4.75	6.00	7.50
19	Half with S. O.	2.75	4.25	5.50	7.25	8.75
	Activated half	2.00	3.25	4.50	6.50	8.00
20	Half with S. O.	2.25	4.00	5.75	7.50	9.00
	Activated half	1.50	3.25	4.75	6.25	7.25

TABLE 4—Continued

NUMBER OF SPECIMEN		DAYS OF REGENERATION				
		1	2	3	4	5
21	Half with S. O.	3.00	4.50	6.00	9.00	C
	Activated half	2.50	4.00	4.75	7.50	
22	Half with S. O.	2.25	4.00	6.00	8.00	C
	Activated half	2.00	3.75	5.50	7.75	
23	Half with S. O.	2.75	4.25	6.00	7.50	9.00
	Activated half	2.25	3.50	5.00	6.25	7.50
24	Half with S. O.	2.50	4.00	5.75	7.75	9.25
	Activated half	2.00	3.25	4.75	6.25	7.50
25	Half with S. O.	1.25	3.50	5.50	7.25	9.00
	Activated half	1.00	3.00	4.75	6.00	7.00
26	Half with S. O.	3.00	5.00	6.50	9.00	C
	Activated half	2.25	3.50	5.00	7.50	
27	Half with S. O.	4.00	6.00	9.00	C	
	Activated half	3.25	5.50	8.25		
28	Half with S. O.	2.75	4.00	5.75	8.00	9.50
	Activated half	2.00	3.25	4.75	6.50	8.00
29	Half with S. O.	1.75	3.75	5.50	7.25	8.50
	Activated half	1.00	3.25	4.25	6.00	7.00
30	Half with S. O.	2.50	4.00	5.75	7.75	9.00
	Activated half	2.00	3.00	4.75	6.50	7.25
31	Half with S. O.	1.50	3.50	5.50	7.50	8.75
	Activated half	1.00	3.00	4.75	6.25	7.25
32	Half with S. O.	3.00	4.25	5.75	7.50	8.50
	Activated half	2.25	3.50	4.75	6.25	7.50
33	Half with S. O.	2.25	3.75	5.50	7.50	9.00
	Activated half	1.75	3.00	4.25	5.75	7.25
34	Half with S. O.	2.00	3.50	5.50	7.75	9.00
	Activated half	1.75	2.75	4.75	6.25	7.00
35	Half with S. O.	2.75	4.00	5.50	7.75	9.25
	Activated half	2.00	3.25	4.75	6.25	7.50
36	Half with S. O.	3.00	4.00	5.75	8.00	9.25
	Activated half	2.25	3.25	4.75	6.50	7.00
37	Half with S. O.	3.50	5.00	7.25	9.25	C
	Activated half	2.25	3.50	5.00	7.50	
38	Half with S. O.	2.75	4.00	5.75	7.75	9.25
	Activated half	2.00	3.25	4.50	6.25	7.00
39	Half with S. O.	2.00	3.75	5.50	7.75	8.75
	Activated half	1.50	3.25	4.75	6.25	7.25
40	Half with S. O.	4.00	6.00	8.75	C	
	Activated half	2.75	4.50	7.50		
Mean for all observations						
	Half with S. O.	2.65	4.05	5.75	7.63	9.00
	Activated half	2.00	3.27	4.67	6.15	7.23

disks which had been subjected to operations similar to those used in the regeneration experiments. The CO_2 production of small circular pieces of subumbrella tissue cut from the near bell margin of disks under different experimental conditions was in every instance shown, from determination with the biometer by Dr. S. Tashiro, to be parallel to the rate of regeneration of the entire half disk from which it had been removed. In another set of experiments, the results for which will be published elsewhere, the half disks upon each of which the appropriate opera-

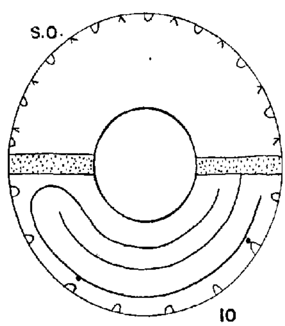

Fig. 10 To show the operation used in experiments of type 6. The sense organs were removed from one-half of the disk, an equal amount of tissue from the margin between the sense organs on the other half and the insulation of the halves brought about by the removal of strips of subumbrella ectoderm. A circuit wave of contraction was maintained in a labyrinth of subumbrella tissue of the half from which the sense organs had been removed.

tion had been performed were kept in separate closed vessels of sea water for the same length of time, and then the comparative amounts of CO_2 produced determined, using normal sea water taken at the time of the beginning of the experiment as the standard of measurement. Here again, as in the tests with the biometer, the total metabolic activity of disks under the several sets of experimental conditions showed the same relation to the presence or absence of the influence of the sense organs as did the differences in rate of regeneration under the same type of experimental conditions.

DISCUSSION

The foregoing data seem to show conclusively that there is a clearly marked influence of the sense organs upon the rate of regeneration in Cassiopea. There is no evidence, however, that

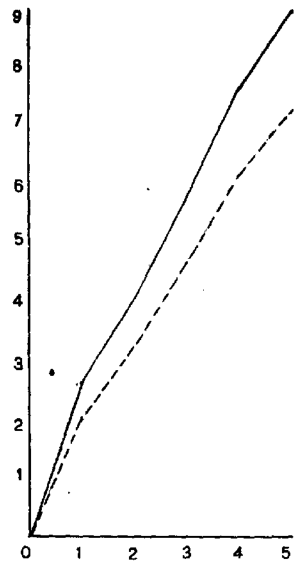

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Fig. 11 Curves to show the comparative rates of regeneration of the halves of 40 disks, on one-half the sense organs remain, while the subumbrella muscles of the other half are activated by a circuit wave of contraction. The upper (solid) line shows the regeneration of the half disks with sense organs, the lower (broken) line the regeneration of the activated half disks. The divisions along the ordinate represent the amount of regeneration in millimeters. Those along the abscissa the time of regeneration in days.

the presence of this influence is necessary for the formationof normal structures in regeneration as Herbst ('96 and '99) concluded from his experiments on decapods; or as maintained by several of the earlier workers upon regeneration in platodes, and by

Walter '11 for Triton. The second of these special cases has been shown by the studies of Morgan, Child, and Goldfarb to be conditioned by influences other than the presence of the nerve centers. The work of Steele has shown also that the removal of an eye stalk is not followed by the regeneration of a heteromorphic structure in several species of crustacea.

While none of these workers has laid any stress on the fact that the nervous system exerts an influence on the rapidity of the early stages of regeneration it has been noted in several instances that the initial stages of regeneration are more rapid in the control animals than in those from which the nervous system has been removed. Thus Goldfarb (op. cit.), page 664, states that in salamanders the hind limb develops more slowly on the side from which the dorsal ganglia innervating the leg had been removed than does that of the opposite side in which the ganglia remained when the spinal cord had already been removed. In a table on pages 665 and 666 he shows that this result was observed in all the specimens recorded save two, in one of which the regeneration was, at the time of measurement, equal for both legs, while in a single specimen the regeneration was most rapid from the side from which the ganglia were removed.

In tadpoles from which the caudal portion of the spinal cord had been removed regeneration of the tail took place more slowly than in the control animals in which the cord was uninjured (p. 672). Again concerning Earthworms from which the head had been cut off and several millimeters of the ventral cord removed he says (p. 708): "The head regenerates rather later in these operated animals than in control animals." In the regeneration of the arms of the starfish (p. 711) a similar observation is recorded.

Goldfarb is, however, of the opinion that "Any severe injury. . . . whether involving the nerves or any other tissue, retards regeneration." Stockard, on the other hand, concluded that in Cassiopea, as Morgan had already shown for a considerable number of animals, the rate of regeneration increased in proportion to the extent of injury and that the deeper the cut—

i.e., the nearer to the center of the disk—the faster would be the following regeneration.

In my experiments the amount of tissue removed from the margin of each half of any disk was the same. The differences in result observed were, therefore, due to the difference in kind not in quantity of tissue removed. As recorded in a previous statement (p. 7) the difference in rate of regeneration is in Cassiopea greatest in the early stages and gradually declines throughout its course, at least through the periods followed in these experiments. Goldfarb's observations appear also to show a similar course of events in earthworms, starfish and amphibians.

This result, on the other hand, is opposed to the conclusion of Child ('10) that "as most experiments not only on the Turbellaria, but on other forms, indicate it is probable that the early stages of the formation of new tissue are largely or wholly independent of the nervous system . . . . ." The later part of the same statement, "but it is difficult to understand how the nervous system of an adult animal could fail to affect the amount and rapidity of growth in an regenerating part composed largely of muscles and sense organs. Absence of such an effect would be in direct opposition to the well established fact of the functional influence of the nervous system on various parts of the organism," is in perfect accord with my results.

Morgulis' ('12) conclusion from his experiments on Brittle Stars that the presence at the cut surface of the radial nerve, either with or without its being in continuity with the remainder of the nervous system, is a "conditio sine qua non" for normal regeneration and that the presence—purely as a mechanical matter—not the functional activity of the nerve is the important factor in regeneration is in direct opposition to my results.

A study of the figures illustrating his paper shows, nevertheless, that the arms in which the nervous connection is undisturbed (control) regenerates most rapidly.

As to the nature of this influence it is evident from the study of the rate of general metabolism of half disks with and without sense organs that it is closely associated with, if, indeed, not identical with, the control of the general metabolism of the animal.

The latter experiments have not been carried far enough to give a definite answer to the question of whether or not there is a gradual decline in the difference in the rate of metabolism corresponding to that shown in regeneration.

This result clearly supports the general contention of Child that the influence of the nervous system on regeneration is indirect, rather than direct, but does not confirm his statement that there is a direct relationship between the rapidity of regeneration and the "characteristic motor activity of the parts concerned." It is shown, that, on the contrary, motor activity may be greatly increased without altering to a proportional extent either the rate of regeneration or general metabolism.

The rate of regeneration appears to be simply one expression of the general metabolic activity of an organism and consequently to be subject to the control of the nerve centers in the same manner as the many other functional activities for some of which, at least, a direct nervous control can not be denied.

SUMMARY

1. The experiments with entire disks, where the rates of regeneration of specimens on which the sense organs remained are compared with those of specimens from which all sense organs are removed, are inconclusive because of wide differences in physiological activity between different individuals.

2. When the insulated halves of a disk, on one of which the sense organs remain, while all of them have been removed from the other half, are compared it is found that the half disk with sense organs always regenerates most rapidly. This is especially noticeable in the early stages of regeneration. The difference in rate falls gradually throughout the course of an experiment (p. 7) (table 1 and fig. 4).

3. When disks prepared as in the experiments mentioned in the previous paragraph are allowed to regenerate in sea water plus 15 parts 0.6 M $MgSO_4$, the regeneration is at first more rapid from the half on which the sense organs remain. Within a few hours the sense organs come under the influence of the anesthetic

and from that time on the rate of regeneration is practically equal from both halves (table 2 and fig. 7).

4. When all the sense organs are removed from a disk and the halves insulated muscular activity may be maintained in one-half by forming an endless labyrinth of the subumbrella tissue and initiating a circuit wave of contraction by induction shocks. Under these conditions the regeneration is faster from the activated than from the inactive half disk. The difference is, however, not nearly so great as when the sense organs are removed from only one of the insulated halves of a disk (table 3 and fig. 9).

5. The comparison of the rates of regeneration of the halves of a disk one-half of which retains its sense organs, while a circuit wave of contraction is maintained in the muscles of the other half, shows that the half disk the muscles of which are contracting under the control of the sense organs regenerates faster, although the rate of pulsation of the activated half is more than three times that of the former (table 4 and fig. 11).

6. The study of the influence of the sense organs on general metabolism—as shown by CO_2 production—has shown that the metabolism of Cassiopea is influenced by the sense organs in a manner quite in accord with the differences in the rates of regeneration under the several sets of experimental conditions.

The influence of the nervous system on the earlier stages of regeneration has been noted by several earlier investigators, but apparently no importance has been attached to it.

These experiments indicate that the rate of regeneration is simply one expression of the general metabolic activity of an animal, and as such is subject to the influence of the nerve centers as are many other functional activities.

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OBSERVATIONS ON AMEBA FEEDING ON ROTIFERS, NEMATODES AND CILIATES, AND THEIR BEARING ON THE SURFACE-TENSION THEORY

S. O. MAST AND F. M. ROOT

From the Zoölogical Laboratory of the Johns Hopkins University

FIVE FIGURES

For several years past we have occasionally collected amebae in a brick-yard pond in the vicinity of Baltimore. These amebae are large lobose forms which correspond, at least superficially, with the ordinary descriptions of Ameba proteus (fig. 1). They feed almost exclusively on living organisms and thrive remarkably well in hay-infusions. When the infusoria in such cultures begin to decline the amebae begin to increase rapidly and frequently become so numerous that the substratum on which they are found appears distinctly grayish; but as the infusoria diminish the amebae decrease in numbers and eventually apparently disappear entirely, but they usually appear again if more hay and water is added so as to induce the infusoria to develop. We have found this to occur in cultures which had been inactive as long as four months. By occasionally adding a little hay and water, we have succeeded in keeping, in ordinary glass dishes, cultures of these amebae for several years. They could, no doubt, be kept indefinitely.

In connection with other work on these amebae we have incidentally observed some remarkable phenomena associated with feeding. Some of these phenomena were of such a nature that it is impossible to account for them on the assumption that movement in ameba is exclusively due to changes in surface tension as is maintained by Bütschli ('92), Ryder ('94), Jensen ('05), Verworn ('09) and others, and they seem to indicate that the rôle played by surface tension is far less significant than has

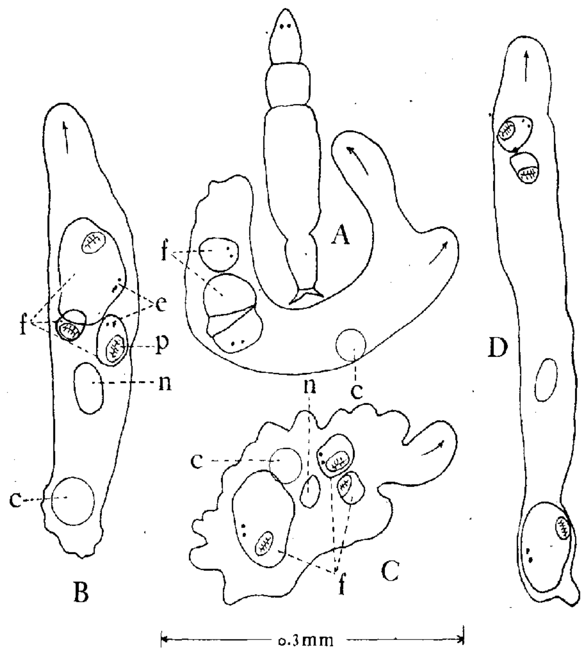

Fig. 1 Camera sketches of a rotifer and an ameba which has been feeding on rotifiers: *n*, nucleus; *c*, contractile vacuole; *f*, food vacuole, containing rotifiers; *e*, eyes; *p*, pharynx; *mm*, projected scale.

A Ameba and rotifier showing their relative size under normal conditions. Drawn November 20, 12 m. Note the large food vacuole in the ameba. It contains one large and two small rotifers, all partially digested and apparently much decreased in size. Only one of the small rotifers is shown in the sketch. Both were attached to the large one when it was captured and they were swallowed with it.

B The same ameba showing the three rotifers in the food vacuoles. Drawn November 20, 12.40 p.m.

C and D Same ameba drawn November 23, 11 and 11.05 a.m., respectively. Note that the rotifers in the food vacuoles have apparently become somewhat smaller during the preceding three days. They also became more translucent, but they were apparently still far from being completely digested. Moreover, when the ameba was discovered they were already much smaller than normal, indicating that they had been captured a considerable time earlier (compare with fig. 2e). Thus it is evident that in this case it would have required much more than three days to digest the rotifers. When the last sketch was made the ameba was active and apparently in excellent condition, but three hours later it was found dead and partially disintegrated.

been maintained by Rhumbler ('05 and '10), McClendon ('12) and others.

Feeding on rotifers. Rotifers belonging to the genus Rotifer were frequently seen in our cultures, sometimes in abundance. In one of the cultures, for more than two weeks, the amebae were found to feed almost exclusively on these animals. Fully half of the specimens examined were either attached to rotifers or contained different parts of them in their food-vacuoles (fig. 1). It seems extraordinary that an ameba should be able to capture and ingest animals relatively so powerful and active as are these rotifers.

The whole process of feeding was not followed through in any given individual, but practically all stages in it were repeatedly observed in different ones. The following account is based upon these observations.

The rotifers in the cultures frequently became attached by means of an adhesive secretion at the posterior end and remained for considerable periods of time. While thus attached the amebae in wandering aimlessly about came in contact with them from time to time. When this occurred they usually surrounded the foot, and to this and the substratum they adhered so firmly that the rotifers could not escape. Soon after an ameba has thus surrounded the foot of a rotifer it begins to flow up around its body. This appears to cause the rotifer to contract sharply forcing the ameba back. After a time the rotifer again stretches out and then the ameba again starts to flow up around it, after which the rotifer again contracts and forces the ameba back. Thus they continue to struggle, sometimes for days (fig. 2). In the meantime the foot of the rotifer begins to digest or liquefy, and this process gradually extends out farther and farther until the animal is killed or until the injury is so great that the contractions and extensions are not strong enough to prevent the ameba from surrounding it. In several cases the rotifers were seen to move considerably after they had been swallowed. This is especially true of young individuals attached to the mother and swallowed with her. In a few cases such individuals were apparently uninjured, for they were seen to swim away after having been released by destroying the amebae (fig. 2).

The process of digestion continues very slowly. Isolated amebae have been seen to retain vacuoles containing rotifers for three days and then the process was not complete. On Novem-

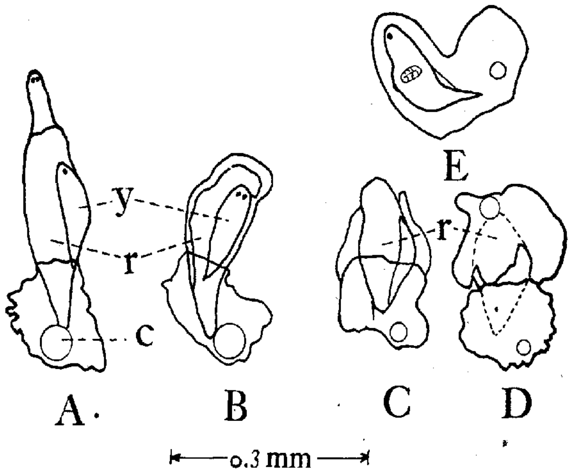

Fig. 2 Camera sketches showing an ameba feeding on a rotifer: *r*, rotifier; *y*, young rotifer attached to the mother; *c*, contractile vacuole; *mm*, projected scale.

A Ameba and rotifer as they appeared when discovered. They had evidently been united for a considerable time for the foot of the rotifer was partially digested. November 30, 2 p.m.

B Same ameba and rotifer, December 1, 9 a.m. The old rotifer was dead but the young one was still alive.

C and D Same rotifer and ameba, also a second ameba, December 1, 12.28 and 1.08 p.m. respectively. At 12.28 the second ameba was rapidly flowing around the rotifier from below; at 1.08 the rotifer was nearly covered, the original. ameba being attached to the posterior and the other to the anterior end. At 2.55 p.m. the second ameba had left the rotifier and the other one was rapidly flowing away. Shortly after this the young rotifier broke through the cuticula of the mother and escaped apparently uninjured. The mother was dead, the cuticula was badly crumpled, and the foot was nearly gone. Note that the rotifer has decreased much in size.

E An ameba containing a rotifer which had just been swallowed. The rotifer was still alive when the sketch was made, although the foot was much injured. The ameba covered only about half of the rotifer when it was found. A little later the pseudopods began to extend rapidly and in a very short time the rotifer was entirely surrounded.

ber 20, 12 m. a specimen containing a large rotifer, already partially digested, was isolated. Three days later the rotifer, although somewhat smaller, was still intact and apparently far from completely digested (fig. 2). During all this time the ameba was kept at room temperature, about 22°, and it was active and in excellent condition. Another specimen containing a rotifer was isolated December 3. Two days later this specimen had divided, but one of the daughters still retained the vacuole containing the rotifer. Similar results were obtained in a number of other cases. Greenwood ('87, p. 284) maintains that rotifers are retained one to three days.

Feeding on nematodes. Small nematodes were found in our cultures at different times but they were never very abundant. These creatures are extremely active. They almost incessantly wriggle about so violently that it seems impossible for an ameba to capture them.

The process of feeding on these creatures was observed in only one case and in this the nematode was more than half swallowed when it was discovered (fig. 3). The anterior end protruded and this was waving about in the most violent fashion. The process of swallowing continued, however, and fifteen minutes later the worm was entirely engulfed. The ameba then became very active, although there was no locomotion; it merely alternately elongated and contracted, extending in nearly opposite directions each succeeding time; and each time it stretched out, the nematode which constantly remained at the posterior end, was folded more closely on itself and forced into a smaller space. Thus the ameba continued to elongate first in one direction then in another until the worm was well coiled up so as to form a rather concentrated vacuole after which it slowly moved away (fig. 3). The ameba was isolated shortly after this time. Two days later it had divided and the vacuole had disappeared. It would consequently appear to require less time to digest these animals than it requires to digest rotifers.

Feeding on ciliates. The following observations were made by Mr. F. M. Root and the description is essentially as presented by him.

When the amebae are feeding on infusoria they assume somewhat the form of a mushroom, consisting of a flat disk-like portion with a serrate edge composed of numerous short pseudopods, supported by a basal portion (fig. 4). When superficially observed the animals, in this form, appear to be perfectly quiet.

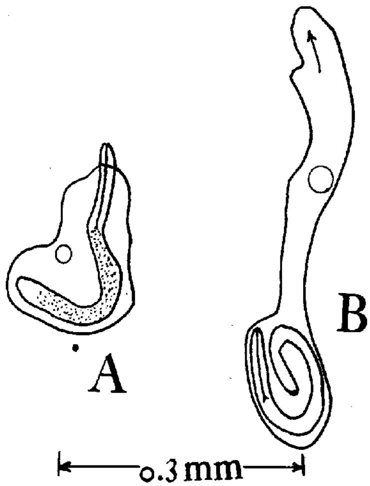

Fig. 3 Camera sketches of an ameba feeding on a nematode: *mm*, projected scale.

A Ameba and nematode as they appeared shortly after they were discovered, December 3, 10.20 a.m. At this time the protruding anterior end of the worm was violently bending back and forth. Fifteen minutes later it was entirely surrounded by the ameba.

B The same specimen, 1 p.m., showing the worm coiled up.

The pseudopods are, however, continuously in a state of flux, alternately contracting and expanding slightly.

In a given culture containing a considerable number of amebae in this form and numerous infusoria I often saw some of the infusoria come to rest and remain quiet for some time in the angle formed between the substratum and the projecting edge of the disk-like portion of the amebae. When this occurred a stout pseudopod usually appeared on either side of the infusorian, also

a thin sheet of protoplasm connecting them above. These continued to flow out over the infusorian, and if it remained quiet long enough it was entirely surrounded and caught. Thus I repeatedly saw amebae capture paramecia, conjugating pairs as well as single individuals, and also some specimens of Stylonychia and Chilomonas.

There is nothing essentially new in the observations described. They are in full accord with those of Blochmann ('94, p. 87); but in connection with them I saw, on two successive days, an occurrence which I have never seen described anywhere, and which I would not have believed possible if I had not seen it. A description of the first case observed follows.

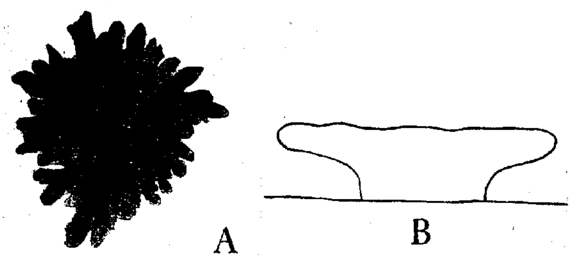

Fig. 4 Photograph and free-hand sketch of an ameba by the junior author, illustrating the form assumed in capturing infusoria.
A Surface view, B Side view.

An ameba, having the form described above, was observed in a watch-glass. A paramecium swam up to it and came to rest with the anterior end in contact with its basal portion. A pseudopod began to project on either side almost immediately as usual (fig. 5a). But when they had reached a point about midway between the two ends of the paramecium, both changed their direction of movement, and flowed directly toward the body of the paramecium, soon touching and then compressing it (fig. 5b). This compression continued until the paramecium was cut in two, one part being taken in by the ameba and the other left outside (fig. 5c). The whole process required only about ten seconds.

During the following hour this same phenomenon was seen to occur in three other amebae; two of the infusoria captured were paramecia and the other was a stylonychia. All of these were observed in the same watch-glass. At this time I hurriedly examined the entire bottom with a binocular and found over twenty half paramecia. Since there were no other organisms present that feed on paramecia, it seems evident that all of those referred to above had been cut in two by amebae. The following

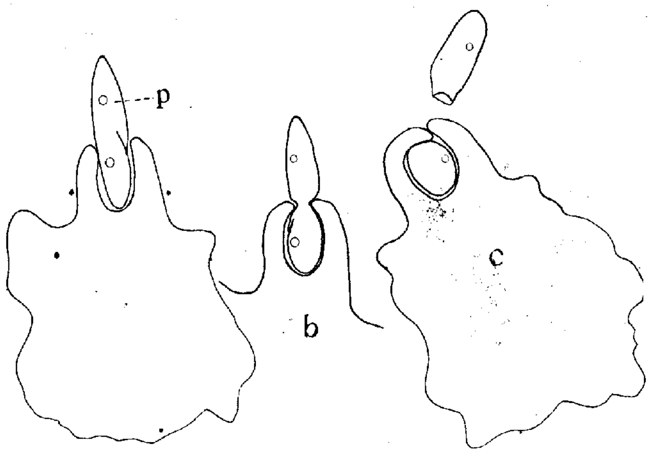

Fig. 5 Free-hand sketches made by the iunior author, showing an ameba capturing and cutting a paramecium in two; *p*, paramecium; *a*, *b*, *c*, ameba.

day this phenomenon was seen in two more cases, the prey being paramecia in both.

A year after these observations were made, one of our students, Mr. H. S. Willis, in connection with a member of the course in biology, made similar observations, but only in a single case. The ciliate captured in this case was not identified, but judging from the sketches and the description made at the time of observation, it probably was one of the hypotricha. The specimen was killed for preservation before the process was complete, the victim being not quite cut in two. In this condition it was seen

by a considerable number of the members of our laboratory. It was, however, later unfortunately lost in the process of staining.

What bearing have these observations on the surface-tension theory? They have a bearing on this theory only in so far as it demands acceptance of the idea that the reactions of Ameba are the result of changes in surface tension.

We have no direct measurements of the surface tension of protoplasm, but on the basis of various indirect measurements and calculations, Czapek ('11, p. 41–43) concludes that it is approximately two-thirds that of water. This conclusion is supported by the facts that protoplasm is rich in organic substances, and that such substances in solution generally tend to lower the surface tension of water. Thus we would expect the surface tension of protoplasm to be considerably lower than that of water.

If this is true, it does not seem possible, a priori, that sufficient power could be developed by changes in the surface tension of a mass of protoplasm like an ameba, either to hold and engulf a struggling nematode or to master a powerful rotifer or to cleave a paramecium. In reference to the last case mentioned we are fortunate in having a method by means of which the question can be put to an experimental test.

The power required to cleave a paramecium can be ascertained with a fair degree of accuracy and from this it is possible to obtain a conception of the magnitude of the surface tension required in an ameba to perform the operation. This was accomplished as follows: One end of a glass rod was drawn out so as to produce a fine flexible fiber about 2 cm. long. A drop of culture fluid containing numerous paramecia was mounted under a binocular. The distal end of the glass rod was now pressed down through the drop. This was repeated more or less at random until a paramecium was caught crosswise near its middle under the end of it. Pressure was then applied steadily until the paramecium was cut in two or the rod bent through an angle of more than 45 degrees.

In this way coarser and finer rods were tested until one was obtained that would cut a majority of the paramecia when bent through 45 degrees. The pressure exerted by this rod was then

measured by applying the end of the rod to the edge of a scale pan and ascertaining the weight necessary to balance the rod when bent through approximately 45 degrees. The results obtained with two of the rods tested are briefly summarized in table I.

TABLE I

DIAMETER OF ROD AT END	LENGTH OF ROD	P. AURELIA						P. CAUDATUM						PRESSURE APPLIED
		Number cut in two			Number not cut			Number cut in two			Number not cut			
		Culture A	Culture B	Total	Culture A	Culture B	Total	Culture A	Culture B	Total	Culture A	Culture B	Total	
mm.	*mm.*													*mgm.*
0.026	20	0	0	0	4	7	11	0	0	0	1	9	10	4.5
0.023	15	4	15	19	3	8	11	0	1	1	2	14	16	9

These results were obtained in tests made with paramecia from two different cultures both of which contained aurelia and caudatum. They are labeled A and B respectively. By referring to the table it will be seen that the results obtained in the two sets of tests which were separated by several days are essentially the same; and it will be seen that none of the 21 paramecia caught under the weaker rod were cut in two, but that 19 of the 30 aurelia caught under the stronger rod were cut in two, while 11 were not entirely cleaved and that only one of the 17 caudatum caught under this rod was divided. In applying the pressure the hand was slowly raised and lowered so as to bring the rod to bear fully upon the paramecium. Some that could not be divided in this way were divided by drawing the rod back and forth across them but these were not recorded as cut. With the small rod, however, we were unable to divide any even in this way no matter how much the rod was bent, showing clearly that the pressure necessary to cleave a paramecium is unquestionably greater than could be applied by means of this rod, that is, greater than 4.5 mgm. The fact that 11 out of 30 could not be divided with the stronger rod without drawing the rod across them would seem to indicate that it is reasonable to accept the pressure produced by

this rod as that necessary to cleave a majority of the animals, and to conclude that amebae in cutting paramecia in two exert a pressure as great as this.

The pressure of this rod bent through 45 degrees equaled approximately 9 mgm. as the following results show. With 9.2 mgm. in one pan the beam could not be brought to a level with the rod applied to the other; with 8.8 mgm. the beam could be brought to a level without bending the rod through quite 45 degrees; while with 9 mgm. the beam became practically level when the rod was bent through 45 degrees.

If now the cleaving of paramecium by amebae in the process of feeding is due to a change in surface tension at certain points, what must the magnitude of this change be? The answer to this question depends upon what actually occurs during the process, that is, upon whether the paramecia are cut in two by the constriction of the rim of a food-cup or by the approach of the distal ends of two pseudopods. We were unable to ascertain from our observations which of these two methods prevailed, but the bulk of the evidence at hand favors the latter. Moreover, if a cup was actually formed some factors aside from surface tension must have been involved in its formation, for it is evident that an invagination is impossible in a body the form of which is dependent solely upon surface tension. Let us however, assume a cup formed and calculate the change in surface tension necessary to account for the cutting of the paramecia on the basis of both of the methods in question.

The surface tension required in case the cutting is due to the constriction of a ring, e.g., the rim of a food-cup, can be calculated from the following formula: $P = \frac{T}{R} ab$, in which P = inward pressure, R = radius of ring, ab, = total area in square centimeters involved in the pressure (P), and T = surface tension per centimeter. Now we know that to cut a paramecium it requires a pressure of approximately 9 mgm. on a glass fiber 0.023 mm. in diameter. The length of the fiber in contact with the paramecium during the process of cutting could not exceed one-half of the circumference. It actually was continuously

much less for as pressure was applied to the fiber the paramecium did not flatten and become wider as was expected, but the depression formed by the fiber extended down either side making it narrower at this region, so that the length of the fiber in contact was at all times less than the diameter of the paramecium and it gradually decreased as the groove made by the fiber became deeper until it was reduced nearly to zero before the paramecium was entirely divided.

Now the larger the area of the fiber in contact, the smaller will be the value of the surface tension in our calculation. We shall, however, make the present calculation on the basis of the maximum, and consider later the effect on the result of reduction due to contraction. The average diameter for 35 specimens of aurelia, the species on which the amebae fed, was found to be 0.049+ mm., nearly 0.05 mm. If we assume this to be the maximum length of fiber in contact in the process of cleaving paramecium, the area in contact will equal (0.005×0.0023) sq. cm. This value may be substituted for ab in the equation given above, and the pressure applied (9 mgm.) for P. Then R in the equation will equal the radius of the paramecium being cut by the ameba. But this varies from 0.0025 cm. to nearly zero, and the greatest pressure required does not occur until after the constriction has proceeded far enough to bring the ectosarc of opposite sides in contact, that is, not until the diameter of the paramecium has been reduced to about 0.001 cm. Assuming this statement to be valid, R would equal 0.0005 cm. Now substituting all of these values in the original equation:

$$9 = \frac{T\ (0.005 \times 0.0023)}{0.0005},\ T = \frac{9 \times 0.0005}{0.005 \times 0.0023} = 391 \text{ grams}$$

per centimeter = 383.18 dynes per centimeter or the minimum tangential tension in the rim of the food-cup required to cleave the paramecium on the basis of the first of the two methods to be considered. But in cutting the paramecium with the glass fiber the maximum pressure was applied when the length of fiber in contact was several times reduced. It is therefore evident that the tangential tension required is greater than 383.18 dynes per centimeter. But in a cup, if the constriction of the rim is

due to surface tension, it is only the difference between the surface tension at the rim and elsewhere that can be effective. If the paramecia then were actually cut by the contraction of the rim of a food-cup due to the action of surface tension, there must have been a difference in the surface tension of the rim of the cup and that of other regions of the ameba equivalent to more than 383.18 dynes per centimeter; and to make this difference possible the surface tension of ameba must have been much larger.

If the cutting of paramecium by ameba occurred in accord with the second method mentioned above, that is, by the approach of the distal ends of two pseudopods, as the bulk of the evidence seems to indicate, and if the cutting quality of the pseudopods was the same as that of the glass fiber, then the pressure exerted by each of the pseudopods must have been approximately equal to 9 mgm. Since the pseudopods and the glass fiber were nearly all of the same size their cutting quality was probably practically the same. We may, therefore, assume that the pressure exerted by them was, if the paramecia were cut in this way, 9 mgm.

If this pressure was the result of the action of surface tension it must have been due to a reduction of the tension at the end of the pseudopods equal to 9 mgm., and the width of the surface involved must have been equal to the circumference of the pseudopods. These were approximately 0.025 mm. in diameter having a circumference of 0.078+ mm. The difference in surface tension between the end and the base of the pseudopods must therefore, if our postulates are valid, have been 9 mgm. or 8.72 dynes per 0.078+ mm., which equals 1118+ dynes per centimeter; and to produce this difference the surface tension of the ameba must have been much higher. Consequently to cut a paramecium in two in accord with this method a much higher surface tension is required than to cut it in accord with the first method.

The process in question would, therefore, require, at the very least, a surface tension considerably higher than 383 dynes per centimeter and in all probability it would require a surface tension higher than 1118 dynes per centimeter, that is, practically

as high as that of molten platinum, the highest yet discovered. But the surface tension of protoplasm is, according to Czapek as previously stated, only approximately 50 dynes per centimeter and with a surface tension of this magnitude in ameba it is questionable whether a greater local reduction than 25 dynes per centimeter could be produced without destroying the organisms. It is therefore evident that surface tension plays a very insignificant rôle in the process of feeding described unless the protoplasm of these organisms consists of some sort of a structure that makes possible a great magnification of the effect of the surface tension. But since there is no evidence of such a structure the required power must, for the present, be sought largely in connection with other phenomena, gelation pressure, absorption pressure, adhesion, cohesion, diffusion, etc.

There is as yet little or no experimental evidence which directly bears upon the relative importance of these different factors or upon the mechanics of their regulation, although it is clear that the reactions in Ameba do not depend solely upon changes in the environment. Movement and changes in movement (responses) may undoubtedly occur without any affective external changes, such responses being entirely due to internal processes. It is also obvious that while some of the responses which are dependent upon external conditions are directly related to the environment, being local responses to local stimulation, others are not; and we are unable to conceive how some of the latter can be explained without assuming that the entire animals are involved as organized systems of considerable complexity, that there are impulses transmitted from one part of the body to another and that there is a regulatory center, in which impulses may originate and in which those originating elsewhere may be modified and controlled. This is especially true regarding much that occurs in the process of feeding on rotifers. It is also true regarding the peculiar mushroom-shape assumed when feeding on infusoria and regarding a considerable number of responses described by Jennings ('04), Kepner and Taliaferro ('13) and others. Moreover, the facts that enucleated parts of ameba do not respond at all or respond in a haphazard fashion,

indicate, as Hofer ('90) concludes, that the nucleus acts as a regulatory center. This conclusion is also supported by the work of Mr. H. S. Willis carried on in our laboratory and now in press in the Biological Bulletin. Gruber ('12) and others, however, oppose this construction, although they obtained results similar to those obtained by Hofer and Willis.

The conclusion reached above regarding surface tension is in harmony with that reached by Jennings ('04) in his observations on the rolling movement in ameba and by Dellinger ('06) in his observations on the walking movement. It is also supported by Kepner and Taliaferro '(13) in their interesting observations on the process of feeding.

SUMMARY

1. Certain amebae at times feed almost exclusively on rotifers, at others they feed largely on paramecia.

2. They capture the rotifers by flowing around the foot at the point of attachment to the substratum. After they have surrounded the foot they begin to flow out over the body. The rotifer responds by contracting and forcing the ameba back, after which it extends again and the ameba again begins to flow out over it, etc. In the meantime the foot begins to digest and gradually the rotifer weakens. Thus they continue sometimes for days before the rotifer is swallowed.

3. When amebae are feeding on paramecia they assume a sort of mushroom shape with a serrate edge consisting of numerous short pseudopods. The paramecia tend to come to rest between and under these pseudopods by which they are usually surrounded, but sometimes the ends of the pseudopods approach each other before they are fully extended and cut the paramecium in two.

4. To cut a paramecium in two with a fine glass fiber it requires a pressure of approximately 9 mgm. If the pseudopods have the same cutting quality as the glass fiber and if their movement is due to a change of surface tension, it requires to perform the work involved, a reduction in surface tension of at least 1118 dynes per centimeter at the tips of the pseudopods.

5. If the ends of the pseudopods fuse so as to take on the form of a ring around the paramecium and if the cutting is due to constriction in this ring, and if the constriction in this ring is due to a change in surface tension, the work involved requires a minimum reduction along the inner surface of the ring of at least 383 dynes per centimeter.

6. The bulk of evidence at hand seems to indicate that the paramecia are divided by the approach of two pseudopods. To account for the process on the basis of the surface tension theory, *therefore, the surface tension of the amebae would have to be,* at the very least, much higher than 383 dynes per centimeter and in all probability considerably higher than 1118 dynes per centimeter. The surface tension of protoplasm is, however, only approximately 50 dynes per centimeter. It is, therefore, probably at best an insignificant factor in the process of feeding in ameba.

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THE EFFECT OF MOISTURE UPON THE SILK OF THE HYBRID PHILOSAMIA (ATTACUS) RICINI BOISD. ♂ × PHILOSAMIA CYNTHIA (DRURY) ♀

ONÈRA A. MERRITT HAWKES

Philosamia ricini and of P. cynthia from Ning-po were crossed in 1914 in order to study the method of inheritance of certain spots on the larva, the colour of the cocoons, and the arrangement of the long white hairs on the abdomen of the moth. The results of the preliminary work on cocoon colour are given in this paper, no reference being made to the still unfinished work on the larva and moth.

When the cocoon colour work was undertaken, it was hoped that the results would help to explain the lack of coincidence between the work of Kellogg and Toyama on the inheritance of colour in cocoons. The breeding, thus far, has thrown no light upon the method of inheritance, but, if the cocoons used by them, are as much affected by moisture as the hybrid here discussed, their results may have to be considerably modified.

Philosamia ricini is found wild in Assam, but has also been domesticated to a considerable extent in that province, and to a smaller degree, in other parts of India. A careful study of the life history has been made at Pusa, India. The form there cultivated is not pure, some larvae being spotted instead of spotless, and some of the cocoons being a pale fawn instead of a pure white. Every effort is, however, made to keep the broods as nearly as possible like the wild form, which has a spotless larva and a pure white cocoon. Owing to the uncertainty of the Pusa stock, the specimen used in this cross was chosen from a batch of white cocoons collected in the Assam forests.

In order that there should be no doubt about the stock, Mr. J. H. Watson and myself, mated males and females of this batch

and reared the offspring, to find that they produced spotless larvae and white cocoons. One seemed, therefore, justified in presuming that the parent P. ricini came from a stock which produced only white cocoons.

P. cynthia differs markedly from P. ricini in that it has, typically, a red-brown cocoon. Owing to some unknown cause, the intensity of colour varies considerably. I have not up to the present been able to rear this species in order to study the variations of intensity and the possible causes which may produce that variation. A related species, P. canningi, also has the cocoon colour varying from a rich red-brown to a pale fawn: this species has been reared, but only for one generation. Experiments were to be made on the second generation to test if moisture had any effect on the intensity of colour, but the moths unfortunately did not pair. It is important to notice that neither P. cynthia nor P. canninghi, is known ever to have produced a white cocoon.

As the result of the crossing, the female, P. cynthia laid about 180 eggs, a large proportion of which were fertile. At the end of three weeks there were 149 larvae, which produced 135 cocoons and from them there emerged 111 moths, only three being cripples. The family was exceptionally healthy in spite of the exotic conditions under which it was grown. The larvae grew quickly and reached a length of $2\frac{1}{2}$ to $3\frac{1}{2}$ inches—as large as any grown at Pusa. There was very little disease.

These cocoons exhibited every variety of fawn and pale brown—none were white, none were the deep red-brown characteristic of P. cynthia. One was therefore, inclined to conclude that the brown colour acted as a weak dominant.

The cocoons (F_1 generation), were spun in the privet leaves on which the worms had been reared or on the nets which covered their cages. The nets were either black or white. The colour of the nets had no influence upon the intensity of the colour of the cocoons, as both darker and lighter cocoons occurred side by side on both black and white nets. One colour phenomenon, however, constantly occurred; when a cocoon was spun in leaf, it was always a darker colour on the side towards the leaf,

the leaf never being large enough to extend entirely around the cocoon.

The second generation of cocoons (F_2) varied much more than F_1 some being a dark red, the majority fawn, and a few so light in colour that one was inclined to describe them as white. These very light cocoons, however, were found, when opened, to have a fawn coloured lining, whereas those of P. ricini which I had reared myself, were white throughout. All the nets used in rearing this generation were black.

Mr. W. Bateson very kindly looked at the cocoons of the F_1 and F_2 generations, and, influenced by his own early work on cocoon colour, advised a very careful study of every possible environmental influence. As a result, hours were spent in watching the larvae spin. It was thus ascertained amongst other facts, that the first silk produced might be either brown or white. I was, however, never successful in collecting this first silk straight from the mouth of the larvae, but have seen sufficient on the glass lid of a cell, to have no doubt of the colour. The brown silk, even when thus seen in some quantity, was a fawn brown, never the deep red-brown which characterised so many completed cocoons.

Bateson had worked at Eriogaster lanestris and Saturnia carpini and concluded that they spun a very light or white cocoon as a result of unhealthy conditions or "unnatural conditions such as disturbance at the time of spinning or removal from the food plant when the growth is nearly complete." That unhealthy conditions had nothing to do with the light cocoons produced in this hybrid is, I think, quite clear from the general condition of the larvae as described above. Mr. J. H. Watson, a well known breeder, inspected the larvae and stated that he had never seen any in better condition. Disturbance, in the case of this hybrid had no influence, as is demonstrated by the experiments presently to be described.

Whilst these general observations were in progress, a number of larvae were being reared, for special breeding purposes, in separate cells. In every case except one, these larvae produced deep brown cocoons. These cells, being filled with privet leaves,

were invariably damp, or even saturated with moisture. The brown of the cocoons was so striking, that a special cause was at once sought. Now, the only difference in the environment of these solitary larvae and those in the large cages was moisture: hence, there seemed a possibility that moisture might be a factor in cocoon colour. In all my experiments the larvae were reared exclusively on Privet (Ligustrum vulgare).

The forests in which the Philosamias live are very moist at certain seasons, but I have been unable to obtain any satisfactory information as to the degree of moisture of these forests, and as to whether the moist seasons coincide with the periods when these larvae spin their cocoons.

In order to test the effect of moisture, the following typical experiments were made.

A. Experiments on unfinished cocoons

1. A larva began to spin a cocoon in a dry room. At 10 p.m. the first threads were a pale brown. The larva was removed from the cage and placed in a dried box. *The next morning, at 8 a.m., the cocoon was complete and a pale brown colour. This experiment was repeated* several times and it was found that if the orignal silk were brown, the cocoon was brown, but not a deep brown. When subsequently exposed to moisture, cocoons made under these conditions, became a deep red-brown.

2. A larva began a cocoon of white silk at 9 a.m. At 10 p.m., the cocoon was white but incomplete; the cocoon was then removed to a dry box containing $CaCl_2$. By 8 a.m., the following morning, a good cocoon was complete, the colour being a dirty white. The cocoon was left in the cell and the pupa died of desiccation.

3. A larva at 9 p.m. had completed a pure white platform. The larva and the platform were then placed in a damp cell with a dozen broken privet leaves. The following day at 10 a.m., the platform had become brown, but it was deserted by the larva, which had spun a complete new brown cocoon. On the evening of the same day, the leaves were removed from the box, the box dried and $CaCl_2$ introduced. After ten days the colour was unaltered, but the pupa had died of desiccation. This larva finally spun a dark cocoon although it was disturbed by me and as a result deserted its original cocoon.

4. A larva began to spin brown silk in a damp cell. The leaves which produced the moisture were at once removed, the cell dried, and $CaCl_2$ introduced. The cocoon was finished and was a pale brown colour. Three months later, when the cocoon was placed in a very damp box, it became much darker.

5. One white cocoon was spun in a damp cell and remained white. Unfortunately it was not possible to breed from the moth which emerged from this cocoon.

B. Experiments on finished cocoons

Cocoons containing larvae

1. On Jan. 26, at 10 p.m., four pure white, completed cocoons were found together on the net. One was placed in a damp cell, leaving a tiny piece of silk outside in the dry room. The following day at 9 a.m. the cocoon in the box had become brown, whilst the piece outside remained white. Of the three cocoons remaining on the net, two were still pure white, but the third had become brownish at the mouth of the cocoon. By 10 p.m. there was no change in the cocoons. The brown cocoon was then placed in a dry cell with $CaCl_2$. When the cocoons were examined on January 28 no change had taken place in the colour. On February 10, on which date the cocoons contained pupae, the cocoons were still the same colour. During March and April, when the moths were being forced to emerge by warmth and damp, the three white cocoons became a pale brown.

2. A cocoon was white except at one end where it was a pale fawn. It was placed in a damp cell, with the fawn end hanging in the dry room. *By the following morning* all the cocoon was a dark brown except the *excluded end.*

3. A cocoon was fawn except a small attachment piece, which was brown; the whole cocoon was placed in a damp cell, and in fourteen days was a dark, dirty brown.

4. A medium red-brown cocoon was opened, when the interior was found to be a lighter colour than the exterior. This cocoon was placed in a damp cell, and after eleven days had become a deep brown throughout, the inside now being the same colour as the exterior. The original and subsequent coloration of this cocoon are an interesting demonstration of the influence of external conditions.

5. All the white cocoons of F_3 generation became various shades of fawn and red-brown when subjected to warmth and moisture.

The pieces of silk used in these observations, were taken, it will be noted, from, (a), fresh cocoons which contained the larva, (b), cocoons which contained the pupa, and (c), cocoons up to four months old. These experiments all suggest that moisture is a factor of considerable importance in the cocoon colour of this particular hybrid. Moisture, again, would explain the phenomenon already noted, that in all cases of cocoons spun in a fresh leaf, the side towards the leaf was always darker, for owing

to transpiration, this side would be more or less moist. It also probably explains why no white cocoons appeared in F_1 generation, as that family was reared in an unusually moist room.

It has been stated above, that the larvae have been seen to begin by spinning either pale brown or white silk, and that when the first silk was brown, the cocoon was pale brown, whether it was spun under moist or damp conditions. Up to the present only one case is known, in which a white cocoon was spun in a cell saturated with moisture. When the white or pale brown cocoons are subsequently placed in a very damp atmosphere, they became more deeply coloured, and thus approximated to or even attained, the colour characteristic of the species.

These then are the problems to be solved—is the original colour, whether it be white or pale brown, affected subsequently by (a), atmospheric moisture; (b), by this moisture plus oxidation; or (c), by an excretion in addition to these two?

If a larval excretion has any influence, there are two possible factors which have to be considered—moisture and a definite colouring matter. Or, the excretion may possibly contain a colourless substance which, indirectly, causes the silk to change from white to brown.

Experiment to test whether the colouration was due to oxidation plus water, or to the effect of water vapour alone

A piece of silk was taken from a pure white cocoon, fixed to the end of a glass rod and plunged into a flask from which all air had been excluded by prolonged boiling, and which therefore contained only water vapour. The piece of silk became brown instantaneously, the rapidity of the change being hastened by the temperature. Other pieces of silk from this same cocoon had been kept in boxes at 60°–65° F. and 75°–85° F. respectively, and all had become brown, the change taking place more quickly at the higher temperature. The degree of colouration varied considerably in both boxes. It appeared that temperature acted solely as an accelerating factor, as equally deep browns were produced at both lower and higher temperatures. No experiments were tried at a temperature below 42° F., as that is the minimum temperature at which the pupa remains healthy.

The above experiments make it quite clear that moisture, by itself, can change white silk to brown and consequently, atmos-

pheric conditions are of paramount importance in determining the ultimate colour of the cocoons. The experiments, however, throw no light upon the innate difference between the white silk of the hybrid which becomes brown in a moist atmosphere and the white silk of the parent Philosamia ricini, which remains white under all variations of atmospheric moisture.

When two cocoons are formed, one white and one brown, side by side on the net, during the same night, there is some difference in the condition of the two which is difficult to explain by the slight difference in atmospheric moisture which would occur in a small, closed room, between 10 p.m. and 8 a.m. There is here, doubtless, a difference in inheritance, such that the one possessed and the other did not, the power to produce brown silk at the particular temperature and degree of moisture, which occurred in that room and on that night. Nevertheless, all the white or light cocoons made thus, side by side with brown ones, became, even after four months, brown when exposed to a very moist atmosphere. It is a matter of regret that the conditions under which the experiments were made, did not admit of constant observations with a wet and dry bulb thermometer.

But, although atmospheric moisture can change white silk to brown, there remains the possibility, that cocoon colour is normally affected by an excretion.

The larvae, just before beginning to spin, pass a large evacuation which consists of the ordinary frass in a drop of greenish liquid. When dry, this liquid becomes a deeper green. Observations were made five or six times a day, to ascertain whether the cocoons were wet or even damp, as if soaked by an excretion, but such were never found, the cocoons were invariably dry. The colour of the cocoons was frequently irregular, thus the body of the cocoon might be a medium brown, but the peduncle, by which it was attached to the branch or wire, might be almost colourless. This condition suggested that an excretion might have reached to the end of the cocoon, but did not extend as far as the peduncle. The constant dryness of the cocoons, however, appears to negative this possibility. The irregularity of the colour was possibly due to the looser arrangement of the silk on

the cocoon, which would allow it to be more quickly affected than the very closely woven silk of the peduncle.

Moisture may possibly affect cocoon colour in a number of Lepidoptera, but, so far, the only record which I have been able to discover, concerns the English moth, Plusia moneta. This species has both white and yellow cocoons and several observers state that the white cocoons become yellow, when damped. I have been unable to obtain any cocoons in order to confirm these observations.

Each fibre of silk consists of a core of fibroin, surrounded by a layer of sericin and that in its turn by a mucous layer. The silk fibres occur in pairs. The fibroin is composed of fibrils embedded in a structureless matrix. In order to demonstrate the chemical difference which presumably exists between the brown and the white fibres, they were stained as shown in the following table. No attempt was made to ascertain the nature of the chemical difference.

Until more is known concerning the chemistry of silk, and its reactions to environmental influences, it is impossible to disentangle its heredity.

My thanks are due to the Natural History Society of Birmingham for a grant in aid of this research, and especially to Professor Wace Carlier, who aided and advised me very considerably in the technical part of the work.

CONCLUSIONS

1. The larvae of the hybrid moths may begin to spin with a white or a fawn silk.

2. In all but two cases, cited in the text (pp. 00), the white or fawn cocoons became various shades of red-brown when placed in a very moist atmosphere.

3. The above change can take place even when the silk is removed from the cocoon and is due to the effect of water vapour and not to atmospheric oxygen.

4. No evidence has been collected to indicate that the colouration is due to any excretion from the larva, and there is some negative evidence suggesting that excretion has no influence at all.

5. Further research is needed to ascertain the chemical differences between brown and white silk in those species which produce both kinds. Further breeding is needed to discover the causes to which these variations are due.

REAGENT	WHITE SILK	BROWN SILK
Picrocarmine.	1. Stains rapidly: the fibroin and sericin staining the same colour but the former of a deeper shade. 2. When treated with strong ammonia for a day the picrocarmine does not stain the silk at once. When the stain does act, the mucous layer appears broken and swollen.	1. Did not stain even after an immersion of 18 hours. 2. (a) When treated with strong ammonia for one hour, the fibre did not take the stain but the sericin became very granular in appearance. (b) When treated with strong ammonia for 24 hours the sericin became reddish and the fibroin a deeper yellow. Some fibres did not stain at all.
Water.	No effect.	Did not dissolve out the colour.
Alcohol.	Transverse striations appear in the mucous layer which is gradually dissolved.	Reduces the colour a little by the end of half an hour but never removes it all.
G. Mann's methyl blue eosin.	After one week the fibre became entirely red.	After one week the fibre becomes red.
Water solution of Giemsa's methylene blue	The mucous and fibroin became wine red, the sericin became blue.	All the fibre becomes blue.
Bordeaux red and Heidenhain's iron-alum haematoxylin.	Transverse sections of this preparation were examined. The mucus was of a very deep red, the sericin had not stained, the fibroin appeared as red fibrils in a grayish matrix.	The fibres become red.
Eosin.	The mucus and fibrils are red, the remainder is unstained.	The fibres stain red rapidly.

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THE GERM CELLS IN ASCARIS INCURVA

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ELEVEN TEXT FIGURES AND THREE PLATES

CONTENTS

INTRODUCTION

This paper presents a study of the development of the germ cells in Ascaris incurva. In a preliminary note (Goodrich '14) I reported the existence in this nematode of a remarkable sex-chromosome complex consisting of eight X-chromosomes

opposed by but a single Y. The earlier work on this material, so satisfactory for a study of the maturation divisions, gave promise that it might likewise be favorable for a detailed study of the earlier stages with especial reference to synapsis and to the history of the X-chromatin. In this respect expectations have not been fully realized. The force of Grégoire's reference to Ascaris megalocephala as "Cet object difficile," is most clear; but nevertheless Ascaris incurva has, for these stages, proved more favorable than the better known form. The results show that the history of the events preceding the maturation of the germ cells in Ascaris, while differing in details from that of other forms, is not exceptional among animals in regard to the interpretation of the process of reduction. Here are also presented observations on dimorphism of the spermatozoa, on the early cleavage stages and on the spindle formations in the heterotypic mitosis as well as on the growth stages. The results set forth in the preliminary report were embodied in the following formulae presenting the cycle of the chromosomes. Autosomes are designated as A and the sex-chromosomes as X and Y.

Spermatozoa		Egg		Zygote		
13A + 8X	+	13A + 8X	=	26A + 16X	=	42 (female
13A + Y	+	13A + 8X	=	26A + 8X+Y	=	35 (male)

MATERIAL AND TECHNIQUE

Ascaris incurva, Rud. is parasitic in the stomach of the sword-fish, Xiphias gladius Linn. The material was obtained during the summers 1913, '14 and '15 at Woods Hole. These parasites have been found in every sword-fish examined, but unfortunately the occurrence of the host in that locality is subject to a marked yearly variation. The animals were dissected in physiological salt solutions or in the body fluid only; the gonads were uncoiled and at once transferred to the fixing fluid. Many fixatives were used; those most successful have been: Hermann's fluid which is excellent for all stages but adapted alone for a study of the spindle fibers; strong Flemming's fluid; the modified

Flemming-urea mixture;[1] Gilson-Carnoy's fluid, Bouin's fluid and for cleavage stages particularly, the alcohol-acetic mixtures. It has been found necessary to adopt a modification of the somewhat laborious methods recommended by de Saedeleer ('12), who divided the thread-like gonads into small pieces from each of which preparations were made. In this work on Ascaris incurva the pieces have averaged 5 mm. in length and in critical areas complete serial sections were obtained. For a study of the spermatogenesis such series have been made from specimens fixed in Hermann's fluid, the Flemming-urea mixture and partial series of limited areas for comparison of critical stages in Gilson-Carnoy's fluid, strong Flemming's fluid and in the alcohol acetic mixtures. For oogenesis a series has been obtained from material fixed in Bouin's fluid and (nearly complete) in the Flemming-urea mixture and for comparisons in Gilson-Carnoy's fluid, strong Flemming's fluid, Hermann's fluid, alcohol-acetic and picro-acetic mixtures.

Heidenhain's haematoxylin, and the saffranin and light green combinations have been most generally employed as stains but many others for special purposes as mentioned in the text have been used. Schneider's aceto-carmine has proved valuable for making temporary and Mayer's alcoholic hydrochloric-acid carmine for permanent preparations of the cleavage stages.

OBSERVATIONS ON CHROMOSOMES

A. *Spermatogenesis*

1. The spermatogonia. The chromosomes in the spermatogonial divisions as mentioned in my preliminary note (Goodrich '14) are closely massed (fig. 3) and metaphase plates require considerable extraction in order to distinguish the individual chromosomes. Figure 2 from such a preparation gives a count of 35 chromosomes including the microsome. Figure 1, a, b are from successive sections of a prophase nucleus showing 34 or 35 chromosomes.

[1] I am indebted to Dr. G. L. Kite and to Dr. C. E. McClung for information in regard to this fixative.

2. Presynaptic and synaptic stages. The seriation of the presynaptic stages cannot be determined from the position of the cells in the testis as various conditions are often found within a single section. From synizesis onward, however, the seriation is perfect and using this as a point of departure it is possible, from considerations of nuclear size and structure to determine the preceding seriation. The resting nuclei (fig. 4), indistinguishable from the similar condition of the spermatogonia, contain chromatin irregularly distributed, not infrequently showing paired granules on parallel linin threads, and a large plasmasome centrally located. The succeeding stage (fig. 5), most clearly seen in preparations from the testis fixed in the Flemming-urea mixture, show more conspicuously the paired chromatin bodies lying on parallel threads which are frequently attached to the plasmasome. These chromatin masses cannot be accurately counted as it is seldom that the chromatin is so sharply concentrated in these bodies as in figure 5, but here they certainly do not exceed the diploid number of chromosomes. At this time the process of leptotenization begins (figs. 6 and 7). The chromatin becomes distributed upon the lengthening threads which are often clearly parallel and bear paired granules, and the process continues until the nucleus becomes so filled as to defy analysis. Contraction begins during the later leptotene and early synaptic stages (figs. 7 and 8), the threads appear more heavy and bear a more uniform distribution of chromatin (fig. 8). The plasmasome moves to the edge of the mass thus indicating polarization. The continuation of this process culminates in a typical condition of synizesis (fig. 9). During contraction, threads, sometimes paired, loop out from the central tangle into the clear surrounding nuclear spaces. Figure 10 shows an early stage in the release of the synaptic knot.

3. Post-synaptic stages. Synizesis is followed by a peculiar pachytene nucleus apparently characteristic of the spermatogenesis of the genus (fig. 11). Heavy ribbon-like threads bearing chromatin at either edge (sometimes granules on opposite edges are conspicuously paired) rigidly traverse the nucleus from the centrally located plasmasome to the nuclear wall;

finer threads are also occasionally observed. In some specimens the pachyteme threads resemble somewhat the condition found in oogenesis (page 68) but this occurs more often in material in which the fixation is manifestly inferior. The pachytene stages do not show a conspicuously looped bouquet stage but this is due to the fact that the looped threads extend to the nuclear wall where their course cannot be followed. Later the rigidity is relaxed (fig. 12), threads become somewhat loosened from the plasmasome and in cross section they show a quadripartite structure, the sections approximating a square and showing a tendency for the chromatin to mass at the corners. The threads next become longitudinally cleft—the parts separating and twisting upon each other thus forming a diplotene nucleus (fig. 13). The double threads sometimes show a second longitudinal cleft. The nucleus then enters a long continued, lightly staining 'diffuse' stage (fig. 14) in which the structure of the threads may only occasionally be observed. Linin threads bearing chromatin irregularly distributed traverse the nucleus, lie on the inner surface of the nuclear wall or upon the plasmasome.

Throughout the growth period the plasmasome increases in size (figure 13 shows a portion only) and the chromatin threads interlace upon its surface. It often stains deeply with haematoxylin but after osmic fixation moderate extraction shows a yellowish tinge. When saffranin and light green are used the saffranin stained threads may be seen upon the surface of the green plasmasome and after Auerbach's acid fuchsin and methyl green mixture, the plasmasome is red, and chromosomes green. During the later stages the threads become relaxed from the plasmasome and it fragments, the parts becoming spherical like drops of some non miscible fluid (fig. 15).

4. The prophases. During the early prophases threads reappear and are paired, twisted and show a progressive massing of the chromatin at paired loci upon the parallel threads as they condense to form the chromosomes (fig. 15). The material is not favorable for a detailed study of the formation of the chromosomes; tetrads appear to form by a second cleft appear-

ing transversely across the parallel threads of the stage of figure 12, but as the relation of these structures to those of the diplotene stage is uncertain, it cannot be assumed that a transverse division of the double prophase threads would be equivalent to a similar division of the quadripartite threads of the preceding stage. After fixation with Hermann's fluid the tetrad structure is not visible in the late prophases (fig. 36). The nuclei show thirteen bivalent, six univalents (seven if the microsome can be identified) and one tripartite group; these representing respectively the thirteen autosome bivalents, seven of the X-elements and the long X-component associated with its mate the Y chromosome. The plasmasome may also be distinguished. The tripartite group is of too regular occurrence to be confused with an approximation of a univalent and a bivalent chromosome and as indicated above the enumeration of the chromosomes will account for all the autosomes and for seven of the X-components; the tripartite group which remains is similar to the long X-component and its mate the Y-chromosome as described below when it appears in the maturation divisions (page 67). Material fixed in Gilson-Carnoy's fluid and considerably extracted clearly shows the compound nature of this typical group (fig. 16). Figure 44 shows the same group from various nuclei,—c, d, e after Gilson-Carnoy's fixative show clearly its nature, in (c) and (d) there appear three segments, in (e) four and in figure 16 five. The Y is usually identifiable as the larger and more compact terminal segment because the segment at one end is too small to represent a single chromosome and also the widening of the longitudinal cleft at this end is characteristic of the unapposed end of the long X-component. Figure 44, a and b are after use of Hermann's fluid, b from the prophase and a as it lies in the metaphase plate showing an attachment of spindle fibers in harmony with the assumption of its true tripartite nature (see page 66).

5. The maturation divisions. Figure 17 shows a polar view of a metaphase plate of the first maturation division. The characteristic X group consisting of eight chromosomes, one the microsome and one longer chromosome, is centrally located and surrounded by a group of thirteen autosomes and the Y-chromo-

some lying opposite the end of the longer X-element. This interpretation is confirmed by an inspection of anaphase figures which I described in the preliminary report (Goodrich '14) as follows:

> Early anaphase figures of the first spermatocyte show most clearly the unequal nature of the separation of the chromosome groups. Thirteen autosomes lying at or near the periphery of the plate divide equally, thus forming two anaphase plates of thirteen chromosomes, typically arranged in a ring except that at a point of one daughter plate a gap is observed, opposite which in the other plate is a fourteenth chromosome (fig. 18, a and c). There remain eight chromosomes lagging in the center of the spindle and arranged in a characteristic plate consisting of six chromosomes of average size, the microsome and a larger long chromosome, arranged in an approximately oval or circular plate with the long chromosome projecting from the periphery (fig. 18, b). As the daughter plates separate, this peculiar group tips, apparently as a unit, so that the long chromosome approaches the gap in the ring of thirteen autosomes. Eventually this whole group passes to the center of the ring and thus the two daughter cells (second spermatocytes) receive respectively 14 and 21 chromosomes. Size relations and position facilitate the identification of the homologous daughter chromosomes of the anaphase plates when these are observed within a single section (fig. 18, a and c). Thus the thirteen autosomes of either daughter ring may be readily identified, and by elimination the fourteenth of one ring unmated in the other. This fourteenth chromosome must therefore be considered as a Y-chromosome mated by that member of the X-group, the long chromosome, which is first inserted into the gap of the one ring, corresponding to the space occupied by the fourteenth chromosome of the other. . . . . . From the foregoing it will be clear that the secondary spermatocyte divisions should be of two classes, one showing 21 chromosomes including the microsome, and other 14. Examination of numerous metaphase plates has proved this in the clearest manner to be the case (figs. 19, 20).

The details of the spindle formation in the unequal division are described in a following section, page 79.

B. The oogenesis

1. The oogonia. No oogonial metaphase plates sufficiently clear to be figured had been found when the preliminary note was written but by further search plates have been discovered similar to those of spermatogenesis which when considerably extracted will show individual chromosomes. Figure 21 shows

one of these plates giving a count of 40 chromosomes—the microsomes are not visible and it is probable that the two large paired chromosomes at the upper edge may be the two large components of the X groups.

2. The growth period and prophase. After a rest period similar to that found in the spermatogenesis the nuclei enter the leptotene stage (fig. 23). This is preceded by a condition (fig. 22) showing parallel threads bearing paired granules but no stage of a degree of concentration comparable to that found in spermatogenesis (fig. 5) has been observed in oogenesis. The processes of transformation of the leptotene to a pachytene condition have been observed:

a. Transition showing contraction or synzesis. Figure 25 shows a contraction figure similar to that found in spermatogenesis and this followed by an early pachytene condition (fig. 26) showing heavy threads with no sign of duality except that they occasionally they are attached to divergent fine threads.

b. Transition not showing contraction. In this case the threads thicken in some unanalyzable way (fig. 24) and pass into the pachytene condition (fig. 27) which follows either mode of transition. Although serial sections were made through this section of the ovary no contraction figures were found. The first mode (a) of transformation was observed in the other arm of the ovary of the same animal; the whole ovary had been fixed at one time in the same fixing bath of the Flemming-urea mixture.

Figure 27 shows a late pachytene stage which already gives a few signs of a longitudinal split of the threads. It will be noted that the pachytene threads are at no time conspicuously double as is in the case in spermatogenesis—the fusion of the threads appears to have been more complete (assuming parasynapsis). The strepsinema stage is not pronounced and the pachytene nucleus changes to a diffuse condition (fig. 28). The threads lose their staining capacity and in a large part become massed about the plasmasome, sometimes hiding it completely, and showing irregular areas of greater concentration of chromatin (fig. 29). A few linin fibers traverse the nucleus and occasionally paired granules may be observed upon them. The history of the plasmosome is similar to that observed in spermatogenesis.

The chromatin threads then relax from their position about the plasmasome and fill the nucleus with a diffuse lightly staining network, but shortly there appear throughout the nucleus deeply staining granules which inspection shows to be frequently paired and lying on parallel threads. Moreover, the ends of a pair are sometimes clearly attached to the respective parts of a double chromatin mass which later history shows to be a half of a bivalent chromosome in process of formation (fig. 30). There are no means of identifying these granules with those described in leptotenization but this condition strongly suggests the reversal of the earlier process. Finally the threads become bare of granules, the paired chromatin masses become the bivalent chromosomes; thus it is that the first maturation separates the paired threads of the earlier prophase. Figure 31; a, b (two successive sections of the same nucleus) is of a prophase nucleus and it may well be compared with the same stage in spermatogenesis (fig. 36) as here all twenty-one chromosomes are bivalent (the quadripartite structure is sometimes shown) in contrast to the male nucleus containing seven univalent, thirteen bivalent chromosomes and one tripartite group.

3. *The maturation divisions.* These have already been described in the preliminary report. Figure 32, a and b show anaphase plates from the first division and figure 35 a metaphase plate of the second division; in both cases there are twenty-one chromosomes including the microsome. Figure 33 a and b shows chromosomes from two successive sections of a metaphase plate of the first division in side view after fixation in Bouin's fluid which is favorable for a study of the forms of the individual chromosomes. Not all chromosomes are figured as they are too closely massed, but all cross shaped forms have been included and it will be noted that there are three large chromosomes and one small one of this type, while in spermatogenesis only two conspicuously large cross forms or double V's are present (figure 18 a and c show the receding V-shaped halves of these chromosomes) so it is not improbale that the third cross represents the long X-component now mated with another of its kind. Figure 34 of the same stage after osmic fixation shows the dividing microsome.

DISCUSSION

A. The sex chromosome complex

It has been pointed out that the XY complex of Ascaris incurva comprises a greater number of chromosomes than any hitherto recorded, the nearest approach being that of Ascaris lumbricoides (Edwards '10) where are found five X elements unmated by a Y, and that of Acholla multispinose (Payne '10) in which case five X components are opposed by a single Y, but in this case the Y is equal to or larger than the combined X-elements. It should be noted, however that the work of Kautzsch ('13) and Geinitz ('15) on the somatic cells of Ascaris megalocephala indicates that the single X-chromosome present in the maturation division may be represented by 8 or 9 elements in the somatic cells. · In regard to mass of X-chromatin relative to that of the autosomes it is more difficult to make comparisons based on inspection of the published figures. In A. incurva the numerical ratio of X-chromosomes to autosomes in the spermatids is as 7 to 13 (disregarding the microsome) or about 1 to 2, and this may be considered as roughly proportional to the mass. This mass ratio may be equalled or even surpassed by that of Protenor where the single X-element is of relatively great size. Among other nematodes thus far examined with the exception of Ascaris lumbricoides the X-element is single and also unopposed by a Y, but the ratio of mass to that of the other chromatin is high (Mulsow '12, Gulick '11, Edwards '12). The possible significance of the large X-groups is discussed below.

The behavior of the multiple XY-complex of Ascaris incurva differs somewhat from that of other such compound groups found among the Hemiptera. In the former, the X-group united by linin fibers, acts as a unit in the first maturation spindle where the unequal division occurs, and a definite portion (one end of the long X-component) as shown by position, structure and attachment of fibers, is mated to the Y-chromosome; in the latter, for example Fitchia, Rocconota, Conorhinus, Prionidus, Sinea, Gelastocoris and Acholla. multispinosa (Payne '09) and Thyanta calceata or Thyanta 'B' (Wilson '11), the

X-components possibly excepting Acholla, do not act as a unit until the time of the second, in this case the unequal, division, moreover, so far as can be judged from the figures the Y is not definitely mated to any one member of the X-group.

The genus Ascaris, as pointed out by Wilson ('11) in the case of Ascaris megalocephala, gives some basis for the suggestion of Stevens ('06) that an unmated X may form by release of X-chromatin from a Y-YX bivalent thus leaving the Y-Y portion to function as a bivalent and the X-chromatin as a univalent chromosome. In Ascaris felis the group is an unequal tetrad, the larger component not being visibly compound; in Ascaris incurva the X-element is clearly compound but still united to the Y-Y portion; Ascaris megalocephala shows an X-element sometimes united and again separate from the Y-Y (?) chromosomes which are in this case recognized as autosomes; while in Ascaris lumbricoides it may be that the separation of the compound X from the Y-Y pair has taken place. In this respect the chromosome complex of the genus Ascaris most closely resembles that of Orthoptera such as Hesperotettix, Anabrus (McClung '05) or Leptnia (de Sinety '01) in the association of the X with an autosome or a Y-Y (?) group.

It may be argued that the condition in the above mentioned Hemiptera—the non association of the Y,—elements with each other and with the X-components is in some way correlated with the disjunction of the XY complex in the second rather than in the first division. Thus in these Hemiptera the X and Y elements are distributed somewhat widely in the same plane of the polar plate of the first maturation spindle and prepared for an equatorial division, while in Ascaris incurva Y and YX are opposed in position for a reducing division. Ascaris megalocephala and Acholla multispinosa may represent intermediate conditions, as in both of these forms there exists before the first division a tendency towards association of X and Y components. In Acholla the union is incomplete and disjunction of these elements occurs in the second division; in Ascaris megalocephala, when the X-element is separate, disjunction may occur in either the first or the second division but its behavior when united is

unknown. Thus it may be that the time of disjunction is determined by the mere mechanical mode of association or non-association of the members of the complex. In one case the X-elements must necessarily follow the distribution of that part of the bivalent group to which they are attached and in the other they may be independent and free to divide equationally.

The occurrence of these curious compound X-groups at once presents the question as to their significance. As pointed out by Wilson ('11), Morgan ('10) and Gulick ('11) the behavior and nature of the X-chromosome aside from its relation to sex determination, also offers an interpretation of sex-linked inheritance, if we may assume that determinants other than those influencing sex are also located in the X-chromosome. That the X-chromosome may often be a compound element is shown by Wilson ('12) in Lygaeus and a similar formation is here described for the long component of the X-group. Here, however, and in the various Hempitera that have been discussed, it is by no means clear that the compound X-group behaves as a unit at any time other than during the reduction division. The separation of the elements in the Hemiptera has been mentioned. In Ascaris incurva it has been noted that during the prophases the X-components are widely distributed in the nucleus and there is no evidence to show that there is any uniform behavior during the second maturation division. Also the maturation divisions in the female do not support this contention; the microsome, the most readily identifiable unit of the female sometimes lies in the peripheral ring of chromosomes in which are also often found the large cross-formed autosomes, indicating that the X-components are not at least centrally located in the plate and that they may be quite irregularly distributed throughout the plate. Thus there is no visible evidence to support the contention that the X-elements may behave in the female as a unit and not undergo along with the autosomes the random assortment of synaptic pairs which Sutton ('03) pointed out as a probable basis of Mendelian inheritance. Therefore, while the fact that the compound group is distributed exactly as a single X-chromosome during the differential division in the

male, will give a basis for the typical mode of inheritance of sex-linked characters, it may also be expected that within the sex-linked group there would occur a redistribution of the characters inherited from the respective parents. This redistribution would be more free than that shown by the 'cross-overs' among the sex-linked characters in Drosophila (see Morgan, Sturtevant, Muller, Bridges '15) because it would be based on a random assortment of chromosomes of the X-group, rather than on a transference of determinants between a single synaptic pair of chromosomes, such as is the basis of the 'chiasmatype' theory.

B. The X-chromatin and the plasmasome

One object of this work as planned was to trace, if possible, the history of the X-chromatin through the growth stages of the spermatocyte; and it had been anticipated that the X-chromatin would be distinguishable as a compact chromatin nucleolus or nucleoli similar to the conditions so clearly demonstrated in many insects—for example; Wilson '05 a, b, '10, '11, '12, Davis '08, and Payne '09. In the preliminary report it was stated that "During the growth stages a part of the chromatin is massed in a large irregular karyosome." This was from observations on material fixed in Gilson-Carnoy's fluid after which this body takes an intense haematoxylin stain. A more detailed study, however, has shown that this is a plasmasome, often entwined or even penetrated by chromatin threads but bearing no resemblance to the chromatic nucleolus of insects. As has been outlined in the description this body takes plasma stains while the threads upon its surface stain as chromatin. A similar body is found in the inter-kinetic stages of the gonial divisions; it reappears in the growth stages and finally fragments during the prophase forming a number of droplets in both oogenesis and spermatogenesis. The mass of the chromatin threads or knots upon its surface in spermatogonesis is in no way comparable to that of the huge X-group nor is there in spermatogenesis, at least, any tendency of the chromatin to concentrate in masses resembling a nucleolus or comparable in number with

the X-elements. There have been found in the nucleus no other bodies that could be interpreted as chromatin nucleoli.

While there can be no doubt as to the presence of chromatin nucleoli and their identity with the X and Y chromosomes in certain insects, this history is by no means so clearly proven for other groups. Most workers on mammalian germ cells have merely mentioned that the nucleolus-like body might be the X-element. For example Wodsedalek ('13 and '14) on the pig and horse, Guyer ('10) and Winiwarter ('12) on man, Stevens ('11) on the Guinea pig and Jordan ('11) on the opposum. The conditions as described in the Guinea-pig and in the opposum are more nearly demonstrative but in no case is the proof so rigorous as in the insects. Among nematodes, Gulick ('11) on Heterakis and Strongilus identifies a nucleolus only in the later growth stages as surely being the X-chromosome; Schleip ('11) on Angiostomum identifies the X-elements in post-synaptic nuclei. Mulsow ('12) figures nucleoli of early growth stages of Ancyracanthus but their history is not traced to the prophases where the X-chromatin seems undoubtedly to exist as a more compact deeply staining body. In Ascaris incurva it may be concluded that the X-chromatin throughout all growth stages exists in a condition indistinguishable from the autosomes.

C. The seriation

It has been emphasized in the record of observations that the growth period of both oogenesis and spermatogenesis of Ascaris incurva may be subdivided into the series of stages that has become so clearly recognized in other classes of animals. To mention a few examples: mammalia, Winiwarter ('00); amphibia, Janssens ('05); fishes, Schreiner ('05); insects, Davis ('08); mollusks, Popoff ('07); annelids, Schreiner ('06); Sagitta, Bordás ('12), etc., and for which the nomenclature suggested by Winiwarter ('00) so generally accepted, has been used in this paper. Thus to summarize:

After the post-gonial resting period the nucleus becomes filled with fine threads—the leptotene stage, followed by a stage of

contraction or synizesis (McClung '05) during which the thick threads are formed—zygotene stage; the thick threads persist for a considerable period—pachytene stage; they are early or even throughout of double nature and later split lengthwise—diplotene stage, lengthening and twisting about one another as this occurs—strepsitene stage. A diffuse, unanalyzable stage enters here which is followed by the prophases of the maturation division. Of these stages, the pachytene nucleus does not so clearly show the looping, characteristic of many forms, and the strepsitene stage as it merges rapidly with the 'diffuse' condition is less readily identifiable; but the series is essentially like that established elsewhere.

No description clearly showing this seriation in Ascaris has hitherto appeared but a critical study of the figures of Hertwig ('90), Brauer ('93) and Tretjakoff ('05) indicates that certain conditions observed by these writers may be correlated with similar conditions in Ascaris incurva so that it is highly probable that the same complete seriation also exists in all species of Ascaris.[2] DeSaedleer ('12) has made a detailed study of the oogenesis of Ascaris megalocephala and correlates his results with this same scheme of seriation but his figures emphasize

[2] That Ascaris megalocephala need not be considered as an exceptional case in regard to the growth stages may be seen by a comparison of conditions there observed with those in Ascaris incurva. The difficulty of correlation has resulted in part from the fact that the leptotene and synizesis stages occur early near the inner end of the long gonad while the cells are quite small.

Hertwig ('90), plate 2, figure 5 and Tretjakoff ('05), figures 7, 78, 79 describe a condition without doubt homologous to the late leptotene or early synizeses stage of A. incurva. The figures suggest a fine meshwork of threads and a plasmasome at one edge of the mass showing the same polarization that is found in these stages only, in Ascaris incurva. Hertwig mentions at this stage the absence of a nuclear membrane and it is true that in A. incurva this structure is recognizable with difficulty. Tretjakoff, only, describes a full contraction figure showing looping threads (fig. 80) but does not recognize its significance, identifying a later stage as comparable with this condition in other forms. Hertwig, plate 1, figure 8, Brauer, figures 15 to 21 and 67 to 81, and also Marcus ('06), in Ascaris canis, figure 2, b. c, d, show the type of pachyteme stage characteristic of the genus and comparable with that in A. incurva, figure 11. Tretjakoff's figure 84 may also be of this same condition but both he and Marcus compare this with the synizesis of other forms, as there exists a tendency for chromatin to mass about the plasmasome, but it certainly is not that condition which so

the difficult nature of the material with which he is dealing and are difficult to interpret.[3]

Upon the basis of his studies Brauer, as is well known, has suggested an interpretation of the maturation in Ascaris whereby both divisions are recognized as being equational and so quite at variance with the more generally accepted conception of reduction. He has found undivided linin threads bearing single granules appearing at a stage which seems to correspond to the post-synaptic pachytene stage of Ascaris incurva; these granules and the supporting threads divide twice forming a doubly cleft thread bearing granules arranged in sets of fours. The threads then shorten to form chromosomes, the clefts representing the planes of division of the chromosomes in maturation. In Ascaris incurva it is found that the thread at an early postgonial stage preceding that described by Brauer are paired, they are paired as they enter synizesis, they emerge as double threads and later become quadripartite—in other words at no time is there observed a condition of univalence and a subsequent equational splitting which is the basis of Brauer's contention. The evidence indicates on the contrary that here as in many other classes of animals the chromosomes unite in pairs (side by side) to separate later, reductionally, in the maturation division.

Of the more recent workers Marcus ('06) on Ascaris canis presents a somewhat unusual theory of maturation while Griggs ('06) working on the oogenesis of Ascaris megalocephala, advo-

often intervenes between leptotene and pachyteme stages and of which Marechal ('07) speaks as "un précieux élément de diagnostic" of this critical stage of transformation. Hertwig, plate 1, figures 9, 10, 11 and Tretjakoff, figures 86 to 90 show nuclei similar to the diffuse stage of A. incurva, figure 14.

[3] Of work on oogenesis that of Sabaschnikoff ('97) is not sufficiently detailed for comparison. De Saedeleer ('12) has made an exceedingly careful study but without clear results. His figure 49, showing fine threads and a plasmosome at one side, resembles the leptotene stage of A. incurva. Figures 57 and 58 show contraction stages. The pachyteme and strepsitene stages are not readily identified. He describes a second contraction stage (figs. 234, 235, 236, 238) which resemble in some degree the stage of massed chromatin threads in A. incurva (fig. 29). Figure 146 shows a possibly similar method of chromosome formation to that of A. incurva (fig. 30).

cates the more accepted theory but in both of these cases the conclusion is based on studies of the prophases or immediately preceding stages and it seems clear that a theory of maturation cannot be convincing unless it includes an interpretation of the processes that take place during the whole growth period. Tretjakoff also supports a theory of normal reduction but, as Gregoire has mentioned, his figures are difficult to interpret and he has made no detailed study of the earliest stages—the leptotene and contraction nuclei, incorrectly, I believe, identifying another stage with this latter phase.[4]

The establishment of the accepted seriation of stages for the genus Ascaris is of significance, for in those forms which exhibit this seriation and which are more favorable for study, the evidence indicates that these stages prepare for a true reduction division, and therefore the presumption is strong, that in other forms less favorable for a detailed analysis but which show a like seriation, the processes are the same.

D. The pro-chromosomes and synapsis

The chromatin bodies of the pro-synaptic nucleus (fig. 5) show a marked resemblance to the pro-chromosomes described by Overton ('05, '09) in Podophyllum. The condition of extreme condensation of the chromatin masses is not often observed in Ascaris incurva, more frequently cells are found in the condition shown in figure 6 which may be compared with a similar stage in Acer, Salmonica, Botrychium (Cardiff '06) or in Calycanthus (Overton '09), showing the chromatin somewhat distributed upon the parallel threads. This indicates either that the pro-chromosome stage of Ascaris incurva is of short duration or that there merely exists at this period a tendency towards the extreme condensation which may not always be realized. The process of change from this condition to the leptotene stage may be analogous to the unraveling of the massive bodies described by Janssens ('01), Davis ('08), Wilson ('12) and others, to form the leptotene threads. In A. incurva, how-

[4] A more detailed review of these studies is given by Geinitz '15.

ever, as among plants, (Cardiff '06, Rosenberg '09, Miyake '05, etc.) and in Hydrophilus (Arnold '08) these bodies are conspicuously paired. That the pairing is significant, based on some relation between the thread and not an optical illusion as has been suggested, is shown by the fact that *granules lie opposite one another on the parallel threads and that opposed granules are of the same size.* It is unfortunately impossible to make accurate counts of these bodies but they in no way approach 70, the number to be expected did they represent a precocious splitting of the chromosomes of the last gonial telophase, but rather they approximate the diploid number and therefore more probably represent the pairing of chromosomes preparatory to synapsis. In this case it is clear that in Ascaris incurva, the evidence favors parasynapsis as a haploid number of chromatin bodies arranged in pairs transform into parallel threads and after synizesis in spermatogenesis the pachytene threads show a longitudinal duality.

It has been noted that there exists a difference in the nature of the pachytene threads in spermatogenesis and oogenesis. In the former the pachytene threads are often conspicuously double, in the latter such a structure is not clear; therefore there may be a more intimate union of threads in oogenesis than in spermatogenesis. Unfortunately little work has been done on the comparative study of oogenesis and spermatogenesis in the same forms by the same workers. Stevens ('03, '05,'10) working on Sagitta describes telosynapsis in spermatogenesis and parasynapsis in oogenesis. King ('07, '08) describes telosynapsis as occuring in the male and probably in the female of Bufo. Arnold, '09, on Planaria finds no important distinction between oogenesis and spermatogenesis. Wilson ('12) has shown that in the case of Oncopeltus two different types of conjugation may take place in one sex, i.e., the autosomes unite by a parasynapsis while the sex chromosomes remain in a massive condition and form no intimate union. He has also pointed out that such conditions might explain the lack of transference of hereditary factors between sex-linked groups as is the case in the male of Drosophila (Morgan '12). It is also

true, however, that non-transference in the male is a characteristic of other groups not sex-linked. This condition suggests that in some cases sexes may differ in regard to the mode of synapsis of all chromosomes. Possibly conditions of more or less intimate union of the parasynaptic threads as observed in Ascaris incurva may be one type of such a differentiation.

MECHANISM OF THE UNEQUAL MITOSIS

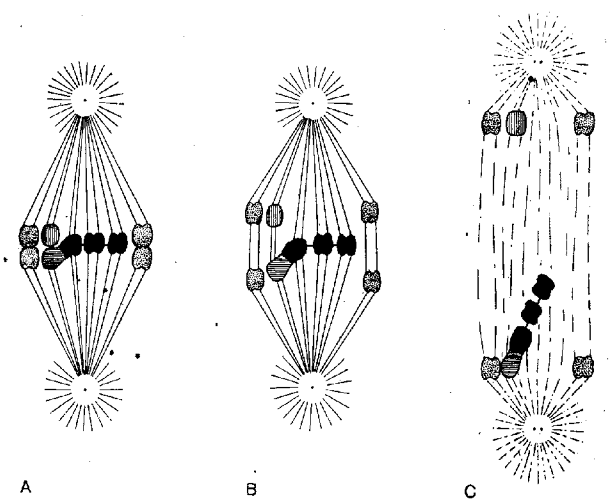
A. Description

A detailed study has been made of the spindle formation of the first maturation division of the spermatocyte with reference to the light it might throw on the problems of mitosis.

Many combinations of plasma and nuclear stains have been tried for this purpose, but Heidenhain's haematoxylin alone or combined with bismark brown or magenta, Delafield's haematoxylin, and saffranin and light green have proved the most valuable.

The early stages are similar to those described by Brauer ('95) for Ascaris megalocephala var. bivalens. The centrosomes separate while lying in the nuclear surface (fig. 36), move to opposite poles, and the astral radiations increase. Spindle fibers then form and penetrate the flattening nucleus and the chromosomes become arranged in the metaphase plate with the X-group centrally located (fig. 37), and bound together by linin fibers. Figure 37, an optical section through the axis of a metaphase spindle, shows the attachment of the spindle fibers. From left to right there may be seen: an autosome bivalent, two univalents of the X-group, the long X-component with the Y above its right end and lastly in the background an autosome bivalent. The attachment of both sex-chromosomes and autosomes is precisely the same; each chromosome is attached at either side to pairs of fibers from opposite poles. The long X-component and the Y are attached as if the group were composed of a bivalent autosome (the Y and the end of the long X opposed to the Y and a univalent, the unopposed end of the long X-component)—that is each of these two units

is joined to two pairs of fibers, one from each pole. This can not often be determined from inspection of a single spindle but compare figure 37 with figure 44, a, which two taken together show the full complement of fibers. This condition is diagrammatically illustrated by text figure A. Here two autosome bivalents are stippled, two X-components and the unmated portion of



Text figs. A, B and C Diagrams to illustrate the unequal mitosis; A is of the metaphase, B of the 'first phase' and C of the 'second phase' of the separation of the chromosomes (see text). Autosomes are stippled, the X-components unmated by the Y-chromosome are in black, the Y-chromosome is vertically ruled and its mate a part of the long X-component is horizontally ruled.

the long X-component are in black and the Y vertically ruled and its mate a part of the long X-component is horizontally ruled.

The process of division may be separated into a first and second phase.

a. The first phase. The peripheral ring of autosomes separates as in a typical mitosis forming two receding parallel

plates united by taut interzonal fibers, but the X-group excepting its long member lies midway of the spindle and parallel to the autosome plates (figs. 38 and 39 and text fig. B). From either pole there proceeds to this group a central core of spindle fibers which with the interfibrillar substance forms a most striking picture that can not adequately be figured. The spindle fibers are attached to either side of each of these chromosomes. Meanwhile one end of the long X-component has retained its place in one of the receding autosome rings (its mate the Y lying in the opposite ring) while the other end remains united to the remainder of the X-group, thus forming a connection between these latter chromosomes and the anaphase plate with which they will ultimately unite. During this process the spindle appears to shorten—the average of measurements of 70 metaphase spindles from centrosome to centrosome is 12.3 microns; that of the early anaphase spindles when the separation of autosomes was not greater than 5 microns, is 11 microns. Later the spindle elongates rapidly to about 18 microns (see note 4, page 82).

b. The second phase. After the stage of figure 38 and 39 a marked change appears, the cleavage furrow appears, the spindle fibers become bent, broken and rapidly disappear (figs. 40, 41 and text fig. C). The X-group is relaxed from its position parallel to the autosome plates and swings as though pulled from the outer end of the long component by the receding autosome plate until it assumes a position parallel to the spindle axes (figs. 41, 42). Figure 40 shows the spindle fibers persisting more clearly than usual and it will be observed that those attached to the X-group are bent and also that a vesicle appears to form about the X-group usually being more noticeable on the side towards the pole from which it is receding. Figure 43 shows the network of linin threads uniting the X-chromosomes. The division is then completed and the chromosomes become rearranged in the equatorial plate of the secondary spermatocyte without entering the resting condition.

B. Discussion

From the foregoing description it is apparent that the motion of the X-group to one pole is determined by its attachment to one half of a bivalent unit (the Y and the end of the long X-component) that divides equally and the X-elements follow or are dragged by that part with which they are united. Otherwise this group is subject to the same forces, be they repulsion or attraction or pull from the centers[5] as are the other chromosomes. During the first phase the seven smaller X-chromosomes are held rigidly perpendicular to the spindle axis as if in a state of tense equilibrium under the influence of equal and opposing forces. As they are non-separable units they remain in this condition in the center of the spindle, not dividing and moving apart like the halves of the bivalent autosomes. During the second phase these forces from the centers do not seem to operate; evidence of tension is gone, fibers become bent, granulated and disappear and the X-group moves from its position as if dragged by the autosome plate to which it is attached. It is true that sometimes straight fibers may be observed attached to the X-group on that side toward which it is to move and bent fibers on the opposite side (fig. 40). It is as if the fibers were pushing and pulling but such forces would tend to resist the motion of the X-group to its position parallel to the spindle axis and it seems better to regard the conditions as due to the effect that this movement would have upon attached but inactive fibers. It is not impossible that the movement of unmated X-chromosomes may in some cases be due to an unobserved attachment to a bivalent group. The description by Kornhauser ('14) of the first maturation division in Enchenopa curvata is suggestive of such conditions.

[5] The shortening of the spindle as mentioned in the description would indicate an attractive force. It is possible that the measurements (all that could be obtained from one testis) are not sufficiently numerous to be significant for such a slight difference. Meek ('15) however has found in Forficula that the spindle is often shorter in late metaphase and early anaphase stages than in early metaphase stages.

It is not intended here to discuss the various theories of mitosis but merely to note the possible application of one or two.

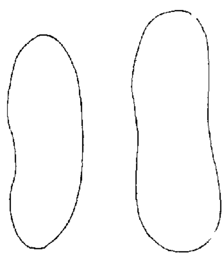
The conditions may be interpreted by the theory of electrical repulsion (Lillie '05)—the metaphase plate being formed by repulsion of the negatively charged chromosomes from the negative astral centers. Here the seven smaller X-chromosomes being univalent remain in the plane of the metaphase plate, while all others due to repulsion of like charges (Gallardo '09) separate and form an extended ring (figs. 18, a and c). This latter formation is such as would be expected from a group of mutually repellent bodies united by fibers, and thus in this case, if it be assumed that these forces operate only during the first phase of mitosis, there does not exist the objection to the theory pointed out by Conklin ('12) in that anaphase chromosomes do not separate as if carrying like charges but tend rather to mass together.

During the second phase the evidence indicates that these forces no longer operate and the movements seem more readily explainable by the vortical cytoplasmic movements or diffusion currents (Butschli '00, Conklin '02) producing a flow outward from the center of the spindle to either pole and thus carrying out the anaphase plates. It may also be noted that the appearance of the cleavage furrow is coincident with this change and this also has been associated with vortical cytoplasmic movements which shift the cytoplasm from the equatorial plane to pass in part through the spindle to either pole (Conklin '02).

SIZE DIMORPHISM OF SPERMATOZOA

Measurements have been made of 600 nuclei of spermatozoa and a marked dimorphism of size is shown. The cells examined were from preparations from the uterus as frequently mature spermatozoa are not found in the testis (Tretjakoff '05, Romieu '11). The nucleus is a thick circular disc. Its position in the cell is variable, a frequent condition is shown in figure 49, a side view and figure 48, an axial view of a spermatozoon. All measurements have been made from one slide (sectioned material) stained in saffranin and light green. The compact nuclei stain

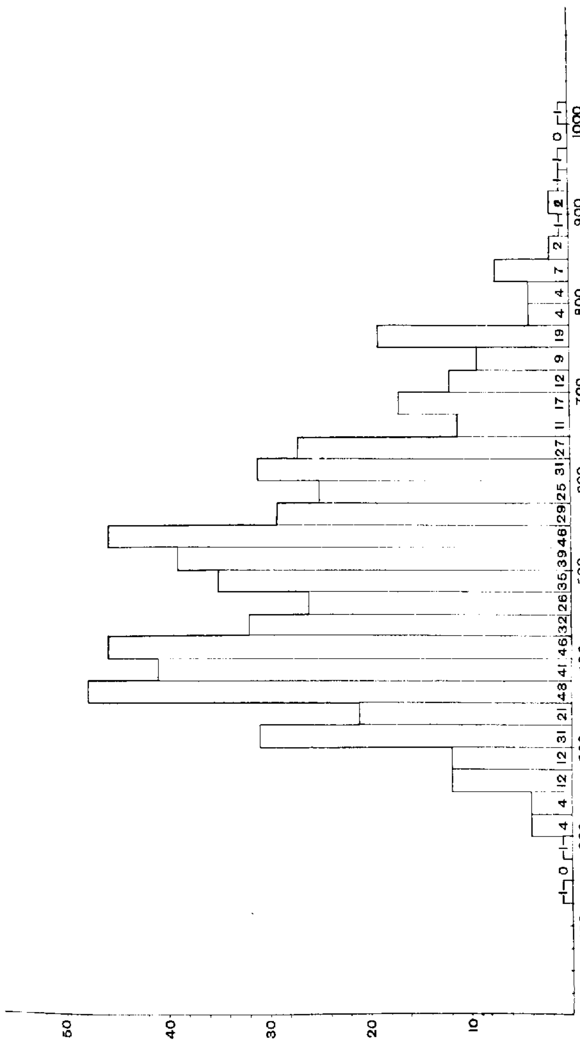
intensely and show clear outlines under the highest magnifications. A 1.5 apochromatic objective, a no. 18 compensating ocular were used and projections made with a camera lucida. Optical cross sections through the center of the nucleus, i.e., such as seen when the disc is observed edgewise were used for measurements as such a section gives data for three dimensions and thus the volume may be computed. To obtain such sections those nuclei were selected which when viewed edgewise were not displaced in apparent position by change of focus, (indicating that the two lateral faces of the disc were parallel to the optical axis) and the greatest optical section thus presented



D

Text fig. D Optical cross-sections of nuclei from the two modal classes.

by each nucleus was projected by camera lucida and outlined. Examples of such projections are shown in text figure D. The length and breadth of these figures were measured by rule to half millimeters. Lengths varied from 13 mm. to 22 mm. and breadths from 5 mm. to 9 mm. From this data index figures proportional to the volume were computed. The radius of the disc is obviously one half of the length of the projection and this squared and multiplied by the breadth gave the index figure. The result is shown in the graph (text fig. E). Vertical distance indicates the number of nuclei measured and horizontal distance the nuclear volumes in terms of the index figures. The curve is clearly of bimodal nature showing that two types of spermatozoa were present. Taking 375 and 525 as modal points we have the proportion 375 : 525 :: 15 : 21 and it will be recalled



Text fig. E A graph showing the classification of 600 spermatozoa of Ascaris incurva, based on estimated volumes of nuclei. Vertical distance indicates number of nuclei measured and horizontal distance, the nuclear volumes in terms of index figures (see text). The number of spermatozoa in a given class is placed above the base line and limiting volumes of classes are indicated below.

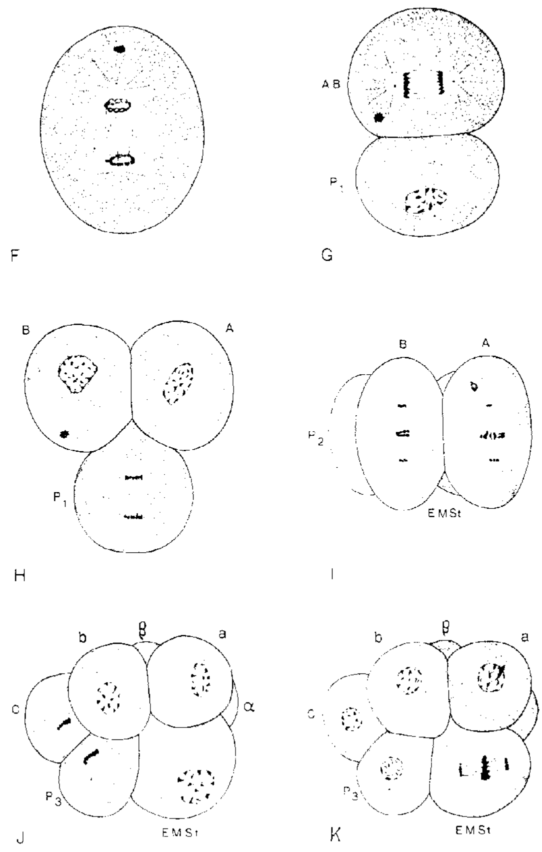
that the two types of spermatids receive respectively fourteen and twenty-one chromosomes. For a more correct estimate of mass the microsome should be omitted, the long X-component given a double value, but this does not change the ratio. Models have been made of the metaphase plates of the second spermatocyte divisions and these have been weighed and give an average ratio of 14 : 21 between the two types. Thus there exists a marked relation between the nuclear volumes and the number of chromosomes contributed to the two types of spermatids. The correlation is close considering the difficulties of measurement and realizing that individual chromosomes vary in size. The correlation of the observed and expected ratios is no more close than those reported by Zeleny and Faust ('15) or Wodsedalek ('13 and '14) but the separation of the modal points is here proportionally much greater.[6] There therefore is less doubt that the bimodal character of the curve is significant and as the expected ratio is also greater and closely proportional to the observed ratio, the results support the conclusion of these writers that the size dimorphism is a result of the chromosomal dimorphism of the spermatids due to the presence or absence of the sex chromosomes.

THE EARLY CLEAVAGE

The cleavage as far as followed proceeds as in Ascaris megalocephala (Boveri '99).

I have been unable to obtain preparations favorable for study of the chromosomes in cleavage. They are closely massed and irregular in form. Chromatin diminution, however, occurs in the somatic cells. Text figures F-K are semi-diagrammatic and based on total preparations, stained in aceto carmine or in alcoholic, hydrochloric acid carmine. These show anaphase spindles of all divisions to the eight cell stage. These show that the diminution occurs as in Ascaris lumbricoides during the third cleavage (Meyer '95, Bonevie '02) rather than as in

[6] Zeleny and Faust ('15) the highest ratio of 35 forms is 1.00 : 1.12; Wodsedalek ('13) on the pig 1.00 : 1.20; ('15) on the horse 1.00 : 1.05. In Ascaris incurva ratio equals 1.00 : 1.40.



Text figs. F to K Cleavage stages in Ascaris incurva. Figures are semi-diagrammatic and are based on camera drawings from total preparations. Anaphases of all divisions to eight cell stage are shown.

Ascaris megalocephala during the second cleavage (Boveri '99). Thus figure F, the first cleavage and figures G and H, the second cleavage show no diminution whereas in Ascaris megalocephala diminution usually occurs in the cleavage corresponding to that shown in figure G of the AB cell. Thereafter as far as followed diminution occurs as in Ascaris lumbricoides—that is in cells A and B as they form cells a and b (fig. I, and in cell EMSt as it forms E and MSt (fig. K), but not in the keimbahn cells, P as it forms EMSt and P_2 (fig. H), P_2 as it forms P_3 and C (fig. J). Figure 46 from sectioned material shows the nature of the process. A relatively large mass of chromatin is thrown out at the periphery of the metaphase plate remaining as an irregular ring about the central core of spindle fibers as the anaphase plates recede from one another. Figure 47 shows a division without diminution.

The P cell and its descendents may be distinguished from other cells by possession of yolk spherules present, apparently in greater quantity than in the corresponding cells in Ascaris megalocephala.

SUMMARY AND CONCLUSION

1. There is present in Ascaris incurva a sex chromosome complex consisting of 8 X-chromosomes and one Y-chromosome, which is mated by a definite component of the X-group.

2. The cycle of the chromosomes may be represented by the formulae:

Spermatozoa	Egg		Zygote			
13A + 8X +	13A + 8X	=	26A + 16X		=	42 (female)
13A + Y +	13A + 8X	=	26A + 8X	+ Y	=	35 (male)

3. The elements of the X-group appear to be mutually independent except during the reduction division.

4. The X-chromosomes are carried to one pole in the reduction division on account of their attachment to one member of a bivalent chromosome unit consisting of the Y-chromosome and its mate among the X-components; otherwise these elements are equally affected by the opposing forces acting during the first phase of the mitotic division.

5. The heterotypic mitosis may be divided into two phases; one characterized by action of equal and opposite forces from the spindle pole, the second characterized by an apparent cessation of the forces and movements probably due to cytoplasmic currents.

6. Measurement of volumes of nuclei of spermatozoa gives a bimodal curve and the ratio between the volumes of nuclei of the modal classes is closely proportional to the ratio between the numbers of chromosomes contributed respectively to the male producing and female producing spermatozoa.

7. The X-chromatin is indistinguishable from other chromatin during the growth stages.

8. The growth stages present a seriation comparable with that of other forms and therefore Ascaris need not be considered as a case exceptional among animals in regard to the interpretation of the nature of reduction.

9. Paired bodies resembling 'pro-chromosomes' are found during the presynaptic stages, which transform during lepto-

tenization into parallel threads and thus enter the contraction figure indicating a parasynapsis.

10. A more intimate union of the paired threads in synapsis is observed in the oogenesis than in spermatogenesis.

11. Elimination of chromatin during cleavage occurs as in Ascaris lumbricoides at the four cell stage rather than at the two cell stage as in Ascaris megalocephala.

In conclusion I should like to express my thanks to Dr. E. B. Wilson for most helpful suggestions and advice during the progress of the work and to Dr. E. G. Conklin and authorities at Princeton University for aid and for facilities afforded during the academic year 1914–15.

FEBRUARY, 1916

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DESCRIPTION OF FIGURES

All figures were drawn with a Zeiss 1.5 mm. apochromatic objective, a no. 12 compensating ocular and projected with camera lucida to table level. Figures as here reproduced give a magnification of 2100 diameters. Fixatives employed are indicated by abbreviations: Strong Flemming's fluid (F); Flemming-urea mixture (F-u); Hermann's fluid (H); Gilson-Carnoy's fluid (G. C.); and Bouin (B).

PLATE 1

SPERMATOGENESIS

1 a, b. Spermatogonial prophase. (*H*) *a* and *b* are two successive sections from the same nucleus.

2 Spermatogonial metaphase (*G. C.*).

3 Spermatogonial metaphase (*H*).

4 Spermatogonial resting stage (*H*).

5 Pre-synaptic stage showing pro-chromosomes (*F-u*).

6 Early leptotene stage (*F-u*).

7 Later leptotene stage (*F-u*).

8 Synizesis (*H*).

9 Synizesis, (later) *F-u*).

10 Pachytene stage (early, release of contraction figure) (*H*).

11 Pachytene stage (later, showing double threads) (*H*).

12 Pachytene stage (still later showing quadripartite division of threads) (*H*).

13 Strepsitene stage (*H*).

14 Diffuse stage (*H*).

15 Prophase showing fragmentation of phasmasome (*H*).

16 Prophase (*G. C.*).

17 First spermatocyte metaphase, showing the X-group centrally located.

18 a, b, c. First spermatocyte anaphase. Figures are from one spindle; a, showing upper ring of 13 autosomes and *Y*; *c*, showing lower plate of 13 autosomes and gap opposite position of *Y* in a; and b, showing the intervening X-element of 8 chromosomes.

19 Second spermatocyte metaphase showing 14 chromosomes (*F*).

20 Second spermatocyte metaphase showing 21 chromosomes (*F*).

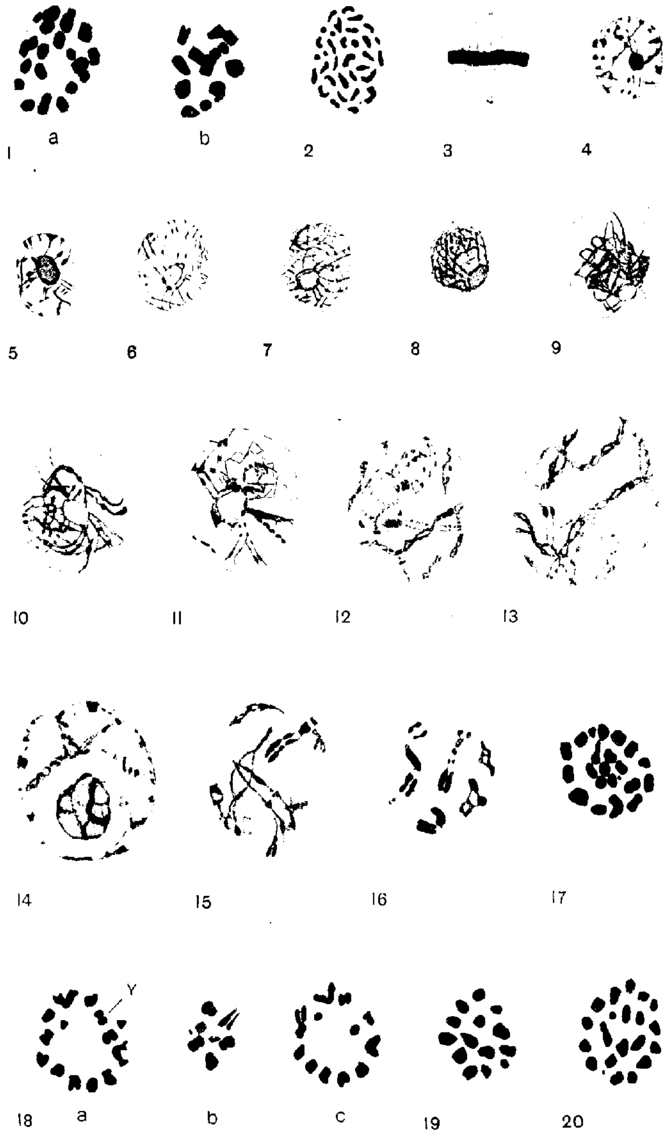

1 a b 2 3 4
5 6 7 8 9
10 11 12 13
14 15 16 17
Y
18 a b c 19 20

PLATE 2

EXPLANATION OF FIGURES

OOGENESIS

21 Oogonial metaphase (*G. C.*).

22 Oogonial resting stage (*F-u*).

23 Leptotene stage (*F-u*).

24 Transition stage (synapsis) not showing contraction figure (*F-u*).

25 Transition stage (synapsis) showing contraction or synizeisis (*F-u*).

26 and 27 Pachytene stages.

28 Strepsitene stage (*F-u*).

29 Diffuse stage (*F-u*).

30 Early prophase (*B*).

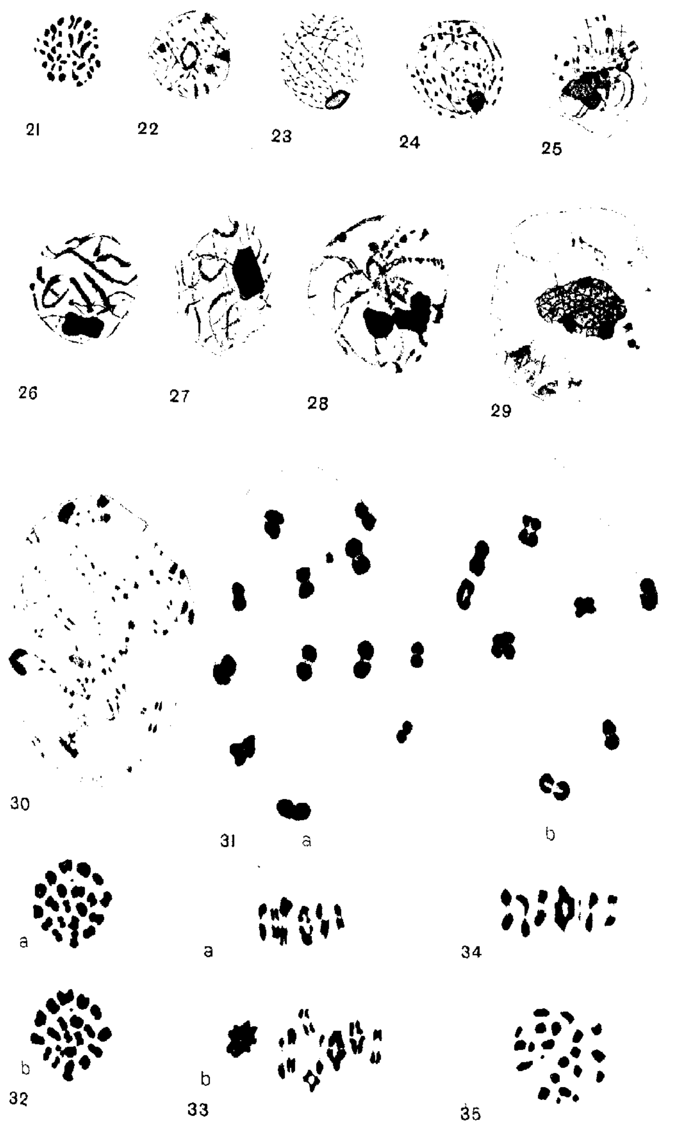
31 a and b. Late prophase showing 21 chromosomes. (Two successive sections of same nucleus) (*F*).

32 a, b. First oocyte anaphase. Daughter plates from one spindle and each showing 21 chromosomes (*G. C.*).

33 a, b. First oocyte metaphase, side view. (Two successive sections from same nucleus and some chromosomes are not figured) (*B*).

34 First oocyte metaphase, side view. (A portion of a plate to show dividing microsome) (*H*).

35 Second oocyte metaphase showing 21 chromosomes (*H*).


21
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a
b
a
a
34
b
32
b
33
35

PLATE 3

EXPLANATION OF FIGURES

36 First spermatocyte prophase, showing 13 bivalents, 6 or 7 univalents, and one tripartite group (*H*).

37 First spermatocyte metaphase (*H*).

38 and 39 First spermatocyte anaphase, first phase (*H*).

40, 41, and 42 First spermatocyte anaphase, second phase (*H*).

43 First spermatocyte anaphase (*G. C.*).

44 The long X-component: a, as associated with the Y-chromosome on spindle; a and b after Hermann's fluid: c, d, and e, after Gilson-Carnoy's fluid.

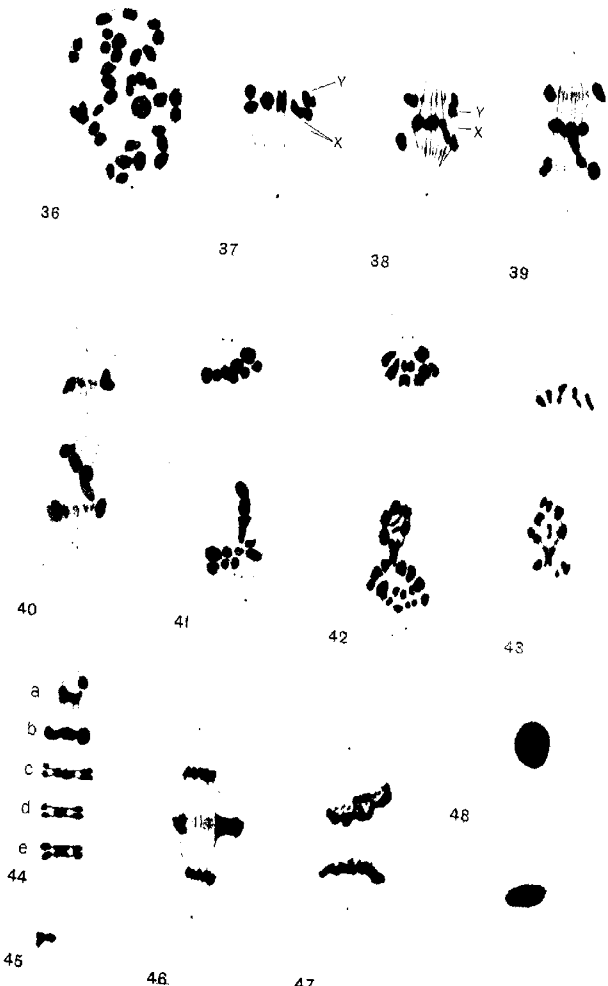
45 Long X-component from second spermatocyte metaphase (*C.*).

46 Cleavage spindle showing diminution of chromatin. Fixed in alcohol-acetic mixture.

47 Cleavage spindle, no diminution of chromatin. Fixed in alcohol-acetic mixture.

48 Spermatozoon, axial view (*F.*).

49 Spermatozoon, side view (*F.*).


Y
X
Y
X
36
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41
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a
b
c
d
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47
49

STUDIES ON THE DYNAMICS OF MORPHOGENESIS IN EXPERIMENTAL REPRODUCTION AND INHERITANCE

IX. THE CONTROL OF HEAD-FORM AND HEAD FREQUENCY IN PLANARIA BY MEANS OF POTASSIUM CYANIDE

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TEN FIGURES

In the sixth paper of this series (Child 13 c) the existence of a metabolic gradient along the chief axis of the planarian body was demonstrated and in the seventh and eighth papers (Child, '14 a, '14 b) the analysis of the metabolic factors concerned in the reconstitution of heads on isolated pieces was begun. In these papers it was shown, first, that pieces of different lengths from different body regions showed different degrees of stimulation and that the greater the degree of this stimulation the lower the head-frequency, and second, that it is determined within a few hours after section and during the period of stimulation of the piece whether a head shall be formed or not.

The relations between length of piece, region of body, degree of stimulation after section and head-frequency constitute one series of data in the analysis of the factors of head-formation. From these data the conclusion was drawn that head-frequency $= \frac{\text{rate } x}{\text{rate } y}$ where x represents the cells directly concerned in head formation and y the other parts of the piece (Child, '14 b).

Another series of data to be considered in this and following papers consists in the experimental alteration and control of head frequency. These data afford a means of testing the formula stated above and throw further light on the problem in other ways.

I. TECHNIQUE

Since considerable numbers of individuals are used in each experiment, some means of standardization of the material as regards physiological condition must be found, and, as noted in earlier papers, size is the best available criterion of physiological condition. In each series, then, animals of the same size from the same stock are used both for controls and for the experimental conditions and with every experimental series a control under standard conditions is made. This is rendered necessary by the fact that the physiological condition of any stock changes with growth, advancing age, temperature, nutrition, etc., and since head-frequency varies with all these conditions a control made at one time is not entirely satisfactory, even for the same stock a few weeks later.

In the experiments described below worms of large size in nearly all cases 16 to 20 mm. long are used and in most cases the region of the first zooid in such worms is cut into three equal pieces, excluding the head. Of course with worms of different size or with pieces of different size the head-frequencies will be different both in controls and in experimental lots, but this factor of size will be considered in a later paper. In animals 15 mm. or more in length the differences in length are due very largely to the growth of the posterior zooids (Child, '11d) the length of the anterior zooid differing only slightly in such animals because it has attained approximately its maximal length, while in smaller animals its length increases with, though less rapidly than, the length of the animal. Except where otherwise stated the comparisons are made with lots of fifty pieces each, a lot including only pieces of as nearly as possible the same size and from as nearly as possible the same level or region of the body and parallel lots being prepared for control and experiment. Experience has shown that this number is sufficient to give fairly definite results and it is about as large a number as can be handled readily. Repetition of the experiments serves as a means of confirmation of the results of single series.

In the experiments with cyanides and various narcotics the pieces are kept in lots of fifty in corked 1 litre Erlenmeyer flasks and the solution or water is renewed every forty-eight hours where the length of time in the solution is more than this. It was determined by experiment that the confinement of fifty pieces in well aerated water in such flasks is without appreciable effect on head-frequency. The controls and experimental lots are kept at the same temperature and under conditions otherwise as nearly identical as possible, except as regards the factor to be tested. The exact method of use of the reagent differs in different cases: the whole animals may be placed in it immediately or some time after cutting. Pieces may be kept in the solution only a few hours or during the whole process of reconstitution, two or three weeks, and of course much higher concentrations may be used for the shorter than for the longer periods. Such details of experiment are stated in connection with each series.

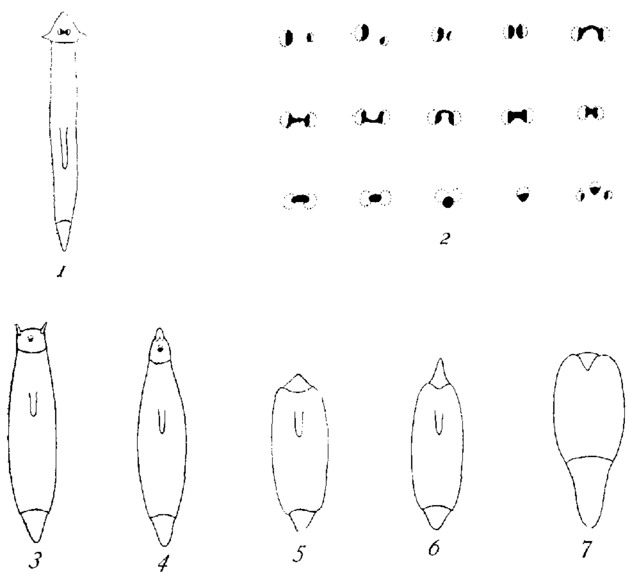
II. THE DIFFERENT FORMS OF THE HEAD

The different forms of head have been described elsewhere (Child, '11 a, '11 b, '15 a pp. 110–112, '15 b, pp. 106–107), but their distinguishing characteristics are briefly stated here.

The normal head is a head like that of the animal in nature, with pointed anterior end, lateral cephalic lobes and two distinct symmetrical eyes (figs. 8 and 9).

The teratophthalmic head is one in which the eyes show some departure from the norm. They may be unequal in size, asymmetrical in position or, as in most cases, show various degrees of fusion of the pigment cups and under certain conditions may differ in number from the normal. Figure 1 shows a common type of teratophthalmic head and figure 2 some of the eye forms. The condition of the eyes is merely an index of the condition of the cephalic ganglia (Child and McKie, '11). In general outline and in position of cephalic lobes the teratophthalmic head is like the normal (fig. 1).

The teratomorphic head (figs. 3 and 4) usually possesses an apparently single median eye which histological examination shows to be double in some cases, and the preocular region is only partially developed or absent so that the cephalic lobes, instead of being lateral are more or less anterior. Various degrees of this condition are found. Figure 3 shows one of the less extreme forms in which the cephalic lobes are more or less



anterior but separated, and figure 4 a more extreme type, where the cephalic lobes are fused at the anterior end of the head and only the duplication of the unpigmented sensory area shows that two cephalic lobes are present

The anophthalmic head (figs. 5 and 6) is without eyes as the name implies. In this form the head appears as merely an outgrowth of new tissue at the anterior end, usually without distinguishing morphological characteristics, though occasionally

fused cephalic lobes may appear at the tip as in the teratomorphic head. Histological examination shows that this outgrowth is really a head, for it contains a rudimentary cephalic ganglion, and its behavior also indicates its characteristic in most cases.

In the headless form (fig. 7) the new tissue simply fills in the contracted cut surface and does not grow out beyond the contour of the piece and no ganglion is present.

These different types of anterior end are in reality somewhat arbitrary groupings of the members of a graded series between the normal head and the headless condition and represent different degrees of development of the cephalic ganglia. In standardized material they occur with a characteristic frequency which depends as I have already shown on length of piece and region of the body from which it is taken (Child, '11 a, '11 b). But this frequency can also be altered and controlled in a great variety of ways both through the physiological condition of the worms and by the action of external factors. In this and following papers some of the methods of control and their results are described.

III. CONTROL AND MODIFICATION OF HEAD-FORM AND HEAD-FREQUENCY BY MEANS OF CYANIDE

The following tables give in percentages some of the characteristic results obtained with KCN, the conditions of experiment being given in connection with the table in each case. Experience has shown that with properly standardized material and conditions the limit of error in regions of the first zooid is not greater than 10 per cent in lots of fifty pieces each and is probably often less than this. In other words, differences of more than ten per cent between control and experimental lots are certainly the result of the experimental conditions and not of uncontrolled factors. Since the differences to which attention is called are usually greater than ten per cent there can be no doubt of their significance. In the region of the posterior zooids the error is greater because the lengths of different zooids differ somewhat in different individuals and a piece from this region

representing a certain fraction of the total body length may be the anterior region of one of the posterior zooids in one case, the posterior region in another, and in still others may include parts of two different zooids and its constitution in these respects is a factor in determining the character of its head.

In the present section the important points of each series and certain differences and resemblances between different series are briefly noted. The general conclusions and their interpretation are reserved for the following section. For the sake of brevity, a change in the head-frequency toward the headless end of the series is termed a downward shift a change toward the normal end, an upward shift in head-frequency.

Series 512, table 1, shows the effect on head-frequency in $\frac{1}{6}$ pieces (a–f, fig. 8) of twenty-four hours in KCN of two different concentrations. In the control I *a–f* the head-frequency

TABLE 1

Series 512. Worms 16–17 mm., in laboratory seven months and twenty days before experiment. I. Control in water; II. Each piece as soon as cut in KCN m/200000 for twenty-four hours; III. Forty pieces; each piece as soon as cut in KCN m/50000 for twenty-four hours

LOTS	NORMAL	TERATOPH-THALMIC	TERATO-MORPHIC	ANOPH-THALMIC	HEADLESS	DEAD
Ia	74	26	0	0	0	0
IIa	82	18	0	0	0	0
IIIa	52.5	47.5	0	0	0	0
Ib	2	78	2	14	0	0
IIb		98	2	0	0	0
IIIb		40	20	35	5	0
Ic	12	50	8	16	14	0
IIc	36	48	0	2	12	2
IIIc	7.5	45	5	20	17.5	5
Id	50	46	0	2	2	0
IId	88	4	2	2	2	2
IIId	50	50	0	0	0	0
Ie	50	50	0	0	0	0
IIe	82	16	0	0	0	2
IIIe	47.5	47.5	2.5	2.5	0	0
If	100	0	0	0	0	0
IIf	98	2	0	0	0	0
IIIf	97.5	2.5	0	0	0	0

shows a downward change from *a* to *b* which are both within the first zooid, while in *c* there is a slight further shift downward, but at the same time an increase in normal heads.

These characteristics of I *c* as compared with I *b* indicate, as will appear later, that *c* includes in many cases a portion of the second zooid. The approximate range in position of the anterior end of the second zooid is indicated in figure 8 by the bracket *m*. In I *d* which represents part of the posterior zooids the head-frequency shifts upwards to a marked degree as compared with I *c*, in I *e* it is also high and in I *f*, the posterior tip of the body it is highest of all.

The effect of KCN m 200000, a very low concentration, for twenty-four hours is slight in II *a*, while in *II b* the shift is somewhat upward, in II *c* still more upward and in II *d* and II *e* also upward to a lesser degree, while in II *f* no effect is apparent. Comparing these changes with those produced in III by KCN m 50000, a concentration four times as high as in II, but used for the same length of time, we find that in III *a* the head-frequency is shifted downward to a marked degree in III *b* also downward—while in III *c* –III *f* no change is produced. In short, the lower concentration has little effect on anterior pieces but shifts head-frequency upward in the more posterior pieces except the last, while the high concentration decreases head-frequency in the more anterior pieces and has little effect on the more posterior.

Series 494, table 2, is a second series of $\frac{1}{6}$ pieces (fig. 8) like Series 512, but showing the effect of forty-eight hours in KCN m 200000. The cyanide lots, II *a–f*, show as compared with the control, I *a–f*, a shift downward in *a*, a slight shift upward if anything in *b*, a very great shift upward in *c*, less in *d* and *e* and no change in *f*.

In both these series in which the animals were cut as nearly as possible into equal sixths excluding the head (fig. 8) the *c* pieces often include a part of the second zooid, somewhat more often in table 2 than in table 1, as the higher frequency in the former indicates. The high level of head-frequency in the *c*

TABLE 2

Series 494. Worms 18 mm., in laboratory six months before experiment. I. Control in water; II. Whole worms in KCN m/200000 five minutes before section, pieces cut in this solution and kept forty-eight hours in same concentration

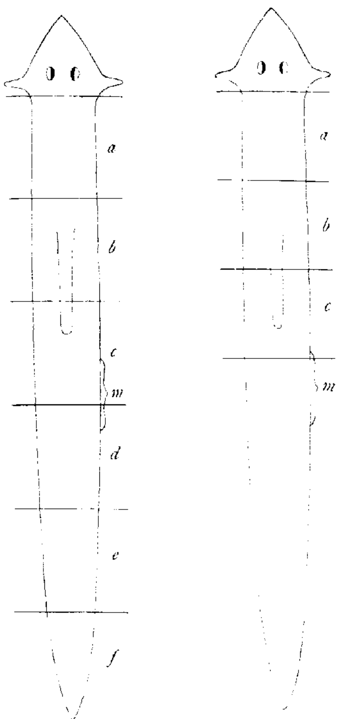
LOTS	NORMAL	TERATOPHTHALMIC	TERATOMORPHIC	ANOPHTHALMIC	HEADLESS	DEAD
Ia	78	22	0	0	0	0
IIa	62	38	0	0	0	0
Ib	0	90	2	4	2	2
IIb	8	92	0	0	0	0
Ic	10	76	0	6	8	0
IIc	60	34	0	4	2	0
Id	28	68	0	0	4	0
IId	72	28	0	0	0	0
Ie	52	48	0	0	0	0
IIe	94	6	0	0	0	0
If	100	0	0	0	0	0
IIf	98	2	0	0	0	0

pieces of the controls as compared with I *c* in Tables 3, 4, and 5, where the *c* pieces are in almost all cases within the first zooid, shows the influence of the posterior zooid region in increasing head-frequency.

The next three series, 489, table 3, 457, table 4 and 432 table 5, include only three pieces, *a*, *b*, *c*, which represent approximately the body of the first zooid. The *c* pieces in these series are usually wholly or almost wholly within the first zooid, although the difference in level of the fission plane in different worms of the same size as indicated by the bracket *m* in figure 9, is such that occasionally these pieces include a portion of the the second zooid. The pieces in these series are then slightly shorter than the $\frac{1}{6}$ pieces of the two preceding series. In the controls I *a–c* of these three series it will be observed that there is a progressive shift downward in head-frequency from *a–c*, a characteristic feature to which attention has been called in earlier papers (Child '11 a, '11 b). The *c*-pieces show a lower level of head-frequency than in Series 512 and 494 above because they are usually within the first zooid.

Series 489, table 3, shows the difference in effect of a given low concentration of KCN when the pieces are cut in it (II)

and when they are placed in it about an hour after section (III). The shift in head-frequency downward as compared with the control is greater in II *a* than in III *a*; in *b* there is no marked



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change in either direction, while in both II *c* and III *c* there is a very great shift upward as compared with the control.

Series 457, table 4, is a comparison of the effect of twenty-four hours (II) and twenty days (III), i.e., the whole period of reconstitution in a given concentration of cyanide. In II the cyanide for twenty-four hours is without effect in shifting the

head-frequency in *a*, shows but little effect in *b*, while in *c* it shifts head-frequency upward to a considerable extent. In contrast to this the same concentration in III acting throughout reconstitution brings about a very great downward shift in *a*, less in *b*, while in *c* there is if anything a slight upward shift. The long period in KCN shows a greater downward shift in *a* and a lesser

TABLE 3

Series 489. Worms 18–20 mm., in laboratory six months before experiment: I. Control in water; II. Whole worms in KCN m/200000 immediately before section, pieces were cut and kept in the same concentration during reconstitution with renewal every forty-eight hours; III. Pieces cut in water, in KCN m/200000 forty-five minutes—one hour and forty-five minutes after section and kept in the same concentration during reconstitution with renewal every forty-eight hours

LOTS	NORMAL	TERATOPHTHALMIC	TERATOMORPHIC	ANOPHTHALMIC	HEADLESS	DEAD
Ia	90	10	0	0	0	0
IIa	24	76	0	0	0	0
IIIa	34	66	0	0	0	0
Ib	0	92	8	0	0	0
IIb	0	86	10	2	2	0
IIIb	0	94	2	2	2	0
Ic	4	38	2	18	38	0
IIc	6	70	4	8	10	2
IIIc	12	66	6	12	4	0

TABLE 4

Series 457. Worms 18 mm., in laboratory two months before experiment: I. Control in water; II. After all pieces cut placed in KCN m/200000 for twenty-four hours; III. After all pieces cut placed in KCN m/200000 for twenty days.

LOTS	NORMAL	TERATOPHTHALMIC	TERATOMORPHIC	ANOPHTHALMIC	HEADLESS	DEAD
Ia	94	6	0	0	0	0
IIa	94	6	0	0	0	0
IIIa	38	62	0	0	0	0
Ib	0	52	4	36	8	0
IIb	0	38	12	26	20	4
IIIb	0	28	6	26	40	0
Ic	4	22	0	16	54	0
IIc	2	40	4	14	40	0
IIIc	0	30	12	12	42	0

upward shift in *c* than the short period. The effect of twenty-four hours in KCN m/200000, as shown in II a–c of this series is essentially similar to the effect of the same concentration for the same time in table 1, II *a–c*.

In series 432, table 5, the effect of thirteen days in two concentrations m/100000 (II *a–c*) and m/200000 (III) is shown. In both concentrations there is a very great shift downward in the *a*-pieces, a slight shift downward in the *b* pieces, more marked in the higher concentration, II *b*, than in the lower, III *b*, while

TABLE 5

Series 432. Worms 16–18 mm., in laboratory twenty-six days before experiment: I. Control in water; II. KCN m/100000 thirteen days with renewal every forty-eight hours; III. KCN m/200000 thirteen days with renewal every forty-eight hours

LOTS	NORMAL	TERATOPHTHALMIC	TERATOMORPHIC	ANOPHTHALMIC	HEADLESS	DEAD
Ia	84	16	0	0	0	0
IIa	6	74	10	4	6	0
IIIa	4	80	6	6	2	2
Ib	10	72	0	10	8	0
IIb	0	56	14	10	20	0
IIIb	0	60	12	16	12	0
Ic	10	28	2	18	42	0
IIc	2	64	8	10	16	0
IIIc	8	78	0	8	6	0

in *c* there is a very marked shift upward, slightly greater in the lower concentration, III *c*, than in the higher II *c*. This upward shift in head-frequency in the *c*-pieces, however, does not increase the frequency of normal heads but merely shifts the head-frequency toward the normal end of the series.

This period of thirteen days in KCN is sufficiently long so that very few or no changes in character of the heads occur after the pieces are returned to water. Since this is the case, this series may be compared in certain respects with Series 489, table 3, and with III, *a–c* of Series 457, table 4, where the pieces were kept in cyanide during the whole period of reconstitution. In general the results are similar in all; a marked shift downward in *a*, a slight effect in *b* and a shift upward but with little or no

increase in normal head-frequency in *c*. In all cases the results of long periods in KCN are in marked contrast to those of short periods both as regards the greater effectiveness of long periods in shifting head-frequency downward in *a*, and their lesser effectiveness in increasing normal head-frequency in *c*. The reason for this difference will appear below.

Certain differences of minor importance between the different long period series appear. First, the decrease in normal head-frequency produced by KCN m/200000 in *a* is considerably greater in table 5, III *a*, than in table 3, II *a* and III *a*, or in table 4, III *a*. Again, the upward shift in head-frequency in *c* is much greater in table 5, III *c*, and in table 3, II *c* and III *c* than in table 4, III *c*. These differences are due to factors not fully controlled in the experiments. My experience with hundreds of such series has shown that inaccuracies in the level of section probably play a larger part in determining such differences than any other factor. Each series is prepared at a single sitting, but I have found that a slightly different habit may be established in different series. In one series, for example, the *c* pieces may average a little larger than the *a*-pieces or vice versa, or the levels of section may differ slightly in different series, so that the *c*-pieces in one series include a part of the second zooid more frequently than in another. On the other hand, the sensitiveness of the material to environmental conditions, which will become increasingly apparent in following papers leaves no doubt that slight differences in temperature, constitution of water or nutritive condition of the worms also play a part in producing such differences in different series. But these differences are not sufficient to obscure the characteristic features and it is a striking fact that different series, of experiments, performed at different seasons of the year with worms which have been kept in the laboratory and have received a food (beef liver) different from the natural food for different lengths of time show so high a degree of similarity.

In series 559, table 6, the effects of periods of different length in KCN m/100000 are compared. The worms used in this series were considerably longer than those of the other series

TABLE 6

Series 559. Worms 20–25 mm., in laboratory one week before experiment. I. Control in water; II–V. Whole worms in KCN m/100000 five minutes before section, pieces cut in this solution and in same concentration after section. II. In KCN three hours; III. In KCN sixteen hours; IV. In KCN forty-eight hours. V. In KCN sixteen days

LOTS	NORMAL	TERATOPHTHALMIC	TERATOMORPHIC	ANOPHTHALMIC	HEADLESS	DEAD
Ia	86	14	0	0	0	0
IIa	72	28	0	0	0	0
IIIa	80	20	0	0	0	0
IVa	74	24	2	0	0	0
Va	0	76	10	10	4	0
Ib	10	50	10	26	4	0
IIb	2	36	14	36	12	0
IIIb	0	46	18	36	0	0
IVb	14	50	12	24	0	0
V	0	34	18	22	20	6
Ic	46	26	6	12	10	0
IIc	34	26	4	24	12	0
IIIc	60	20	4	14	0	2
IVc	74	18	2	4	0	2
Vc	24	74	2	0	0	0

and the first zooid had in many cases undergone a further physiological division (Child, '11 d) so that the anterior end of the new posterior zooid thus formed was almost at the level of the mouth. In such animals the *c*-pieces, cut as in the other series, usually include a part or nearly all of this short zooid and we find correspondingly that the head-frequency shifts upward instead of downward from *b* to *c*. A comparison of I *b* and I *c* in table 6 shows this difference and a further comparison of these percentages with those of I *b* and I *c* in tables 3, 4, and 5 shows the influence of this zooid in increasing head-frequency. The *c*-pieces in tables 1 and 2 which often include a portion of a posterior zooid also show the influence of this region in a higher level of head-frequency as compared with the *b*-pieces.

From table 6, I *a*–IV *a*, I *b*–IV *b*, it is evident that periods of 3, 16, and 48 hours in KCN m/100000 have little or no effect in shifting head-frequency in the *a*- and *b*-pieces. In the *c*-pieces, however, a period of three hours has no effect (II *c*), but

sixteen hours (III *c*) produces a marked shift upward, and forty-eight hours (IV *c*) a still greater shift upward.

For the sake of direct comparison the effects of a long period in the same concentration of KCN are given in V *a–c*. The long period is very much more effective than any of the short periods in shifting head-frequency downward in the *a*-pieces. In the *b*-pieces there is also a shift downward but much less marked than in the *a*-pieces. In the *c*-pieces, however, the long period (V *c*) shows a marked shift upward from the headless opthalmic and teratomorphic to the teratophthalmic but as in the long periods of preceding series, there is no increase, in this case a decrease, in normal head-frequency. Here then, as in preceding series, the characteristic difference in the effect of short and long periods appears.

IV. DISCUSSION

The most important results obtained from the data of the preceding section are briefly as follows: first, in the controls the head-frequency shifts downward as the level of the piece becomes more posterior within the first zooid, but shifts upward again in the posterior zooids and is very high at the extreme posterior end of the body. Second, KCN in low concentrations if it acts for a sufficiently long time shifts the head-frequency downward in the anterior region of the first zooid, produces comparatively little change in the middle region and shifts head-frequency upward except in the extreme posterior region of the body. Third, short periods, up to a day or two in KCN are much less effective than long periods in shifting head-frequency downward in the anterior regions of the first zooid (*a*-pieces) but are more effective than long periods in shifting head-frequency upward in the posterior regions of the first zooid (*c*-pieces).

These rather remarkable results demand analysis. Considering for the present only the first zooid, since the same reagent shifts head-frequency in opposite directions in different regions of the same body, it is evident first, that the factors concerned

in determining the presence or absence and the type of head in the different regions are either quantitatively or qualitatively different. Second, since all the different types of head may arise from any level of the body and since there is every reason to believe that the action of KCN is primarily quantitative, not qualitative, it seems probable that quantitative rather than qualitative differences in the factors concerned in head-formation are concerned. Third, the fact that under natural conditions the head-frequency is highest in the most anterior regions and progressively lower in more posterior regions together with the fact that KCN shifts head-frequency downward in the anterior regions and upward in the posterior regions, suggests the existence of a factor retarding or inhibiting head-formation which is least effective in the most anterior regions and becomes more effective as the level becomes more posterior. If we suppose that cyanide inhibits or retards the process of head-formation on the one hand and the action of the inhibiting factor on the other it becomes possible to conceive how it might shift head-frequency downward in one case and upward in another. Fourth, the fact that short periods in KCN are less effective than long in shifting head-frequency downward in anterior regions and more effective than long in shifting it upward in the posterior regions suggests alternative possibilities. A primary stimulation followed by depression has been observed by some authors with very low concentrations of cyanide and it might be supposed that in Planaria the effect of the short periods is to some extent the effect of this primary stimulation. A moment's consideration shows, however, that this cannot be the case. The primary stimulation, if it occurs in the concentrations used, certainly does not extend over a period of twenty-four or forty-eight hours and it is evident from observation that the activities of the pieces are distinctly retarded by these lengths of time in KCN. Moreover, periods of less than twenty-four hours have but little effect on head-frequency and periods of twenty-four to forty-eight hours do not shift head-frequency upward in the *a*-pieces. Finally, the effect of the short periods differs from those of the long only in degree and there can be

no doubt that the action of KCN in the long periods is inhibitory for the process of reconstitution is greatly retarded and the motor activity of the pieces is greatly decreased. The effect of the short periods as compared with the long cannot then be the result of a primary stimulation, but must be in general inhibitory like that of the long.

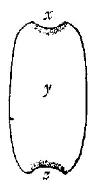
The second possibility is that the short periods act chiefly on the factor inhibiting head-formation which must then be a factor acting rapidly following section of the piece, while the long periods act on the more gradual processes in the cells which give rise to the head, i.e., on the process of head-formation itself. As will appear below, the facts support this hypothesis of a differential inhibition as the basis of the effect of cyanide on head-frequency.

These considerations bring us back to the conclusions reached in the seventh and eighth papers of this series (Child, '14 a, '14 b). In the first of these two papers it was shown that pieces are stimulated by section, the degree of stimulation differing according to size of the piece and region of the body from which it was taken. This relation between degree of stimulation, size of piece and region of body is briefly as follows: short pieces from any level are more stimulated than long, posterior pieces within the first zooid are more stimulated than anterior, and the degree of stimulation of posterior pieces as compared with that of anterior pieces increases as their length decreases. In pieces of one-sixth or less of the total body-length the posterior pieces in the anterior zooid are so much more stimulated than the anterior pieces that their rate of metabolism after section is usually actually higher than that of anterior pieces and remains so for several hours, although before section the posterior region of the first zooid has a much lower rate than the anterior region.

In the eighth it was shown that the determination whether a head shall form or not on a piece occurs within a few hours after section and during the period of stimulation of the piece and it was further pointed out that head-frequency shifts downward or decreases directly as the degree of stimulation of the piece by section increases. These facts suggest that the stimu-

lation of the piece by section exerts in some way a retarding or inhibiting effect on head-formation and development.

In the same paper (Child, '14 b) it was also pointed out that section of a piece is followed by two distinct reactions, the one, stimulation to a greater or less degree of the piece as a whole, the other the reaction of the cells adjoining the cut surface or surfaces, a process of dedifferentiation, division, growth and new differentiation. Leaving out of consideration for the present the posterior cut surface of the piece we may distinguish the region which undergoes dedifferentiation and gives rise to new tissue as x (fig. 10) from the rest of the piece, y, which is temporarily stimulated. The relation between the frequency of head-formation or of the different types of anterior end developed from the region x and the degree of stimulation of the region



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y may then be expressed in a general way by the formula head-frequency $= \frac{\text{rate } x}{\text{rate } y}$. This formula makes no pretensions to mathematical accuracy but is merely a brief way of stating the facts.

It is necessary now to proceed somewhat further in the analysis of this relation between rate x and rate y. In order to give rise to a new head the cells at x must first lose their differentiation and approach or attain the embryonic condition and to undergo this change they must become to a large extent physiologically independent of other parts of the piece and they must also be able to grow at the expense of other parts of the piece. The removal of parts anterior to the cut surface and the presence of the cut surface itself are effective in all cases in inducing more

or less dedifferentiation and growth, but the greater the degree of stimulation of the region y the greater the degree of inhibition of these changes in x and vice versa. As experiments already described have shown, this inhibiting influence of y upon x depends in some way upon the relative rates of metabolism in y and x. The dedifferentiation and division of the cells at x in reaction to the wound and the absence of more anterior parts brings about a progressive increase in metabolic rate in these cells and the stimulation of y also determines a temporary increase of rate in its cells. The stimulation of y undoubtedly occurs chiefly through the nervous system and constitutes what we may call a functional stimulation, *i, e,* it tends to maintain and intensify both the dynamic and chemical correlative conditions which determine and maintain the differentiation of the cells affected by it. The region x, however, is subjected to conditions which bring about dedifferentiation and a return or an approach to an embryonic condition, in other words the cells react to the absence of correlative factors which previously determined and maintained their differentiation.

It is not difficult to see that these two factors are opposite in character, the one tending to maintain the existing differentiation and the other to destroy it. If the functional stimulation of y is sufficient, i.e., if the metabolic rate in y is sufficiently high as compared with that of x, the process of dedifferentiation, and renewed division and growth of the cells of x is retarded or largely inhibited and the cells merely close the wound and differentiate as the correlative conditions originating in adjoining parts of y determine. If, on the other hand, the functional stimulation of y is slight the cells of x are but little affected by the correlative conditions in y and are therefore free to react to the absence of correlative factors from more anterior levels by dedifferentiation, division and growth. Under these conditions a mass of new embryonic tissue is formed and this gives rise to a new apical end or first of all to a new cephalic ganglion, this being, as I have pointed out (Child, '11 c, '13 b, '15 b, pp. 96–116, 188–192), the fundamental action of the specific protoplasm and independent, at least in its earlier stages, of correlative

conditions in other parts of the individual. In short, the formation of a new head on an isolated piece of Planaria, or for that matter, of any other form is a process determined primarily by the constitution of the embryonic cells concerned and not by correlative conditions in other parts. The fact that a head arises directly at the cut surface, whatever the level of the body from which the piece is taken is of itself very strong evidence that this is the case, for if correlative factors in the piece determined the differentiation of the new tissue we should expect that the first parts to appear at the anterior end would be those which in nature are next anterior to this level and that as these are successively formed the head would finally appear. As a matter of fact, however, the head appears first and the other parts arise by redifferentiation of regions of the piece into more anterior regions under the influence of the new head. In short, the piece does not determine the formation of a new head at its anterior end although it may retard or inhibit the process. The developing head, however, does determine the reorganization and redifferentiation of other parts of the piece into those regions of the body which normally lie between the head and the level of the piece. The influence of y upon x is merely negative or inhibitory, while the influence of x upon y is positive and determining. I have pointed out elsewhere (Child, '11 c, '14 b, '15 b) how these facts fall into line with the general conceptions of the axial gradient and of physiological dominance and subordination. Stating the case in terms of these conceptions, we may say that if rate y is sufficiently high y dominates x, inhibits its independent development as a head, and if its dominance is sufficiently complete, which is rarely the case in Planaria dorotocephala, may even determine the development into a posterior end. If, however, rate x is sufficiently high in relation to rate y, x becomes the dominant region, develops independently of y and determines its redifferentiation.

Turning now to the cyanide experiments we see that they are not only readily interpreted on this basis but afford very strong evidence for this conception of the process of reconstitution. KCN as a reagent which depresses metabolism, inhibits to a

greater or less degree, according to concentration and metabolic rate in the cells, both the processes in the head-forming cells of x and the stimulation in y. In those pieces where the stimulation of y is slight, as in the a-pieces, the inhibiting action of the KCN on x appears directly as a shift downward in head-frequency. In the regions where the stimulation of y is great, as in the c-pieces the effect of the KCN in inhibiting this stimulation overbalances its effect in inhibiting x, consequently the head-frequency is shifted upward.

The differences in susceptibility of the regions x and y to cyanide are factors in determining these opposite effects of cyanide. The relation between susceptibility to KCN and various other reagents and conditions has been considered at length elsewhere (Child, '13 a, '15 a, Chap. III). In general the primary susceptibility varies directly with metabolic rate but in very low concentrations such as were used in the experiments recorded above the higher the metabolic rate the more rapid and complete the acclimation. Moreover, the higher the metabolic rate, the more rapid and complete the recovery after a short period in KCN. Both acclimation in KCN or other depressing agents and recovery afterward consist in a gradual increase in metabolic rate. In the case of KCN, however, neither acclimation nor recovery is complete even with concentrations as low as those used in the above experiments.

In the piece of the planarian body the region x which undergoes dedifferentiation sooner or later acquires a higher metabolic rate than the region y, consequently its primary susceptibility to KCN is greater than that of y, but its ability to become acclimated in KCN or to recover after it is also greater than that of y. KCN decreases metabolism in both x and y but x acclimates or recovers more readily and more completely than y. In the a-pieces then, where y has little inhibiting influence on x the shift downward in head-frequency represents the direct inhibiting effect of the KCN which has not been compensated by acclimation or recovery. In the c-pieces, on the other hand, the shift upward in head-frequency results from the fact that the inhibition of stimulation of y by KCN over-balances the

direct uncompensated inhibiting effect on x which appears only in the lesser upward shift and the lower frequency of normal heads in the longer periods. In the b-pieces the two effects of the KCN are almost balanced, so that little change in head-frequency occurs. From this point of view it is easy to account for the difference in effect between short and long periods in KCN. The short period of a day or two is as effective as a much longer period in inhibiting the stimulation of y but has much less effect upon the gradual changes in x than the long period because only the earlier stages of these changes occur during the short period and these are retarded by the KCN so that most of the change in x occurs after removal to water and recovery and is therefore much less inhibited than when it occurs in KCN.

In the a-pieces where the stimulation of y is slight and has but little effect in inhibiting head-formation the short period in KCN is much less effective than the long in shifting head-frequency downward, because recovery in x after the short period is much more complete than the acclimation of x in the cyanide. The difference in head-frequency between the control and the long period a-pieces in cyanide represents the metabolic difference between standard natural conditions and acclimation of the x region to the cyanide.

In the c-pieces, on the other hand, where the stimulation of y is sufficiently great to inhibit head-formation in a large percentage of cases, the short period is just as effective as the long in inhibiting this stimulation and at the same time inhibits the change in x to a less degree than the long. Consequently in the c-pieces the short period in KCN produces the greatest shift upward in head-frequency, while with the long period the effect on the x region plays a larger part. This effect on x is evident in table II c, III c, and table 6, V c, where the head-frequency in general is shifted upward as compared with the control, while the frequency of normal heads remains the same as or lower than in the control and much lower than in the short period lots.

In general the higher the concentration of the KCN the more uniform its effect in shifting head-frequency downward. This feature of the action of KCN is indicated only in table 1 where the higher concentration, m/50000, produces a marked shift downward in the anterior pieces and no change in the posterior pieces. Still higher concentrations produce a shift downward in all pieces. In all such cases the effect is of course produced by the direct action of the cyanide on the x-cells, the cells concerned in head-formation. The higher concentrations inhibit these cells to a greater degree than the lower and there is much less acclimation or recovery, consequently with such concentrations this direct effect on the head-forming region overbalances any indirect effect in inhibiting the stimulation of the y-region and the head-frequency shifts downward in all cases. Only where the concentration is so low that a high degree of acclimation or recovery of the x-region occurs is it possible to shift head-frequency upward by means of cyanide.

As regards the region of the posterior zooids, I have found that pieces from this region are in general much less stimulated by section than pieces of the same length from the posterior region of the first zooid. This difference is due first, to the partial physiological isolation of the posterior zooids from more anterior regions (Child, '11 d) and second, to the slight development of physiological dominance (Child, '11 c, '15 b) within each zooid. In consequence of the lower degree of stimulation after section, the head-frequency of pieces from the posterior zooids is much higher than that of the c-pieces of the anterior zooids as shown in tables 1 and 2 above. But the region of the posterior zooids is not entirely independent of more anterior regions and the more posterior levels of any zooid are to some extent subordinate to the anterior region of that zooid. Consequently, as I have found, some degree of stimulation follows section even in these pieces, except perhaps in the most posterior region of the body which is the most completely isolated physiologically of all. In accordance with this fact we find that short periods in cyanide shift head-frequency upward to some extent in all except the extreme posterior pieces from this region (tables

1 and 2, II *e*, II *e*, II *f*), unless the concentration is too high (table 1, III *d*, III *e*, III *f*). This shift in head-frequency is, however, almost entirely a shift from teratopthalmic to normal which represents much less physiological change than a shift from anophthalmic or headless to teratopthalmic. In other words, the change produced by KCN in the relation $\frac{\text{rate } x}{\text{rate } y}$ in these pieces is much less than in the short period *c*-pieces of the first zooid in tables 3 to 5. The extreme posterior piece (*f*, tables 1 and 2) has normally a head-frequency of 100 per cent or nearly and this cannot be shifted upward by cyanide and, as in the *a*-pieces short periods have little effect in shifting it downward while long periods produce a marked shift downward.

In short, all the facts are in complete agreement, the differences in metabolic condition, the differences in head-frequency and the different effects of low concentrations of cyanide in both short and long periods on pieces from different regions of the body all afford evidence for the same conclusion, viz., that head-frequency $= \frac{\text{rate } x}{\text{rate } y}$ and there are no conflicting or contradictory data.

Another possible factor which may play a part in determining rate *x* must, however, be mentioned, though at present I see no way of demonstrating experimentally whether it is concerned or not. It may be that the differences in metabolic rate at different levels of the body, i.e., of the axial gradient (Child, '12, '13 b, '13 c, '15 b), determine intrinsic differences in the rate of reaction of the cells which constitute the region *x* of a piece and which give rise to a head when a head is formed. The experimental difficulty in testing this possibility lies in the fact that the region *x* is associated with a region *y* which inhibits this reaction to a greater or less degree. The only possible way of testing it would be to cut pieces so short that they consist only of the region *x*, i.e., so short that the whole length of the piece is involved in head-formation. It is possible to approach this condition in this species of Planaria but not

to attain it with the degree of uniformity necessary for experimental test. It will probably be possible to make the test in certain other forms, but I have not yet had opportunity to do so. But if intrinsic differences exist in the cells of different levels of the body they can be only quantitative in their effect in this species, for heads of all types may arise from any level of the body under proper conditions, i.e., the cells of all levels are intrinsically capable of giving rise to any type of head and the particular type of head in any case is determined by the relations between x and y, which, as I have shown, can be altered and controlled experimentally. In following papers other methods of control of head-frequency will be considered.

In conclusion, attention may be called to one other point, viz., that the various types of head resulting from the action of KCN are the same as those determined by the differences in size of piece and region of body. There is no indication of any specific action of KCN or any other agent or condition on head-formation and this fact will become increasingly evident in following papers.

V. SUMMARY

1. By means of low concentrations of potassium cyanide it is possible to alter and control experimentally the character of the head formed and the frequency of head-formation in pieces of Planaria dorotocephala. In general, cyanide shifts the head-frequency downward in pieces representing the anterior third of the first zooid while it has little effect on the middle third, and if the concentration is not too high, or the period of action too long, it shifts the head-frequency upward in the posterior third.

2. Short periods in cyanide are more effective than long in shifting the head-frequency upward in posterior pieces and less effective than long in shifting it downward in anterior pieces.

3. The facts indicate that two factors are chiefly concerned in determining whether a head shall develop on a piece or not. These factors are: first, the reaction of the cells adjoining the cut surface, a process of dedifferentiation division and growth

and so the formation of a mass of embryonic tissue from which the head develops; second, the stimulation of the piece as a whole following section, which lasts only a few hours but which, if sufficiently great, retards or inhibits the reaction of the cells adjoining the cut surface.

4. The effects of cyanide in altering head-frequency are due to its action on these two factors. In cases where the stimulation following section is slight, the direct effect of cyanide on the head-forming cells appears in a shift downward in head-frequency, while in cases where the stimulation is great the inhibition of this stimulation by cyanide may shift head-frequency upward if the concentration is not too high or the period of action too long.

5. All the facts at hand indicate that head-frequency varies directly with the metabolic rate of the cells concerned in head-formation and inversely as the metabolic rate of other parts of the piece. It is possible to express these relations by the formula head-frequency $= \frac{\text{rate } x}{\text{rate } y}$ where x represents the cells from which the head develops and y the other parts of the piece.

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FACTORS AFFECTING MALE-PRODUCTION IN HYDATINA[1]

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ONE FIGURE

CONTENTS

INTRODUCTION

After it had been well established that certain agents reduce the number of male-producing females in the families of the rotifer Hydatina senta, two important related lines of investigation were undertaken. One sought to explain how these agents operated to prevent the appearance of male-producers; the other had for its object the discovery of means of increasing the number of male-producers. The former line of research has proven nearly fruitless; the latter has met with some success. The experiments described in this paper were aimed at the solution of these two sets of problems.

[1] Contributions from the Zoölogical Laboratory of the University of Michigan.

EXPERIMENTS ON THE MECHANISM OF THE PREVENTION OF MALE-PRODUCTION

Osmotic pressure

The list of substances which effect a reduction in the number of male-producing females includes ammonium salts, sodium hydroxide, beef extract, manure solution, creatin, urea, and some others. In substances of such widely different properties it is difficult to select a common feature to which their common effect upon the life cycle of the rotifers could be attributed. One possibility that early suggested itself was that the osmotic pressure of the solutions produced the effect observed. Were this the only cause of the reduction in the number of male-producers, it would be expected that those solutions whose osmotic pressure was the highest would reduce male-production the most. Unfortunately it is probably quite impossible to say whether a solution produces osmotic effects in living protoplasm unless that protoplasm changes volume. Tables of osmotic pressures are therefore of little value in determining whether, in the experiments referred to, the reduction in the number of male-producers was proportional to the osmotic effect of the agent employed. As methods of detecting change of volume of the living rotifers or their tissues were found impracticable, it was necessary to resort to conjecture. In the experiment first to be described, a substance was selected which, if the tissues of the rotifers behaved as theoretically perfect semipermeable membranes, would give an osmotic pressure considerably higher than that which obtained in the other solutions used. The substance selected was cane sugar.

Experiment 1. Two sisters isolated January 13, 1911, became the parents of the two lines of this experiment, one of which was reared continuously in sugar solution, the other in distilled water. Food and other conditions were the same for both lines. A $\frac{M}{3}$ solution of cane sugar was kept in stock. It was heated daily to prevent fermentation, and was tested at intervals for inversion to reducing sugar. Once when Fehling's solution was reduced, the stock solution of sugar was rejected and a new one prepared. This stock solution was diluted to $\frac{M}{33}$ for use in the experiments.

The effect of the sugar solution, as shown in table 1, was to reduce the number of male-producers. But the amount of reduction was less than was expected on the theory that osmotic pressure was responsible for that reduction. It was less than the effects of certain ammonium salts whose effects should have been, theoretically, less than those of the sugar.

TABLE 1

Two lines of the rotifer Hydatina senta derived from sisters, one line reared in distilled water, the other in a $\frac{M}{33}$ solution of cane sugar. The number of male-producing (♂♀) and female-producing (♀♀) females is recorded for each line. The cane sugar reduces the number of male-producers

DISTILLED WATER				$\frac{M}{33}$ CANE SUGAR SOLUTION			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	Jan.				*Jan.*		
1	15	3	20	1	15	2	16
2	17	4	16	2	17	1	22
3	19	3	17		17	0	9
4	21	4	21	3	19	3	33
5	22	5	29		19	1	25
6	24	2	26	4	21	4	20
7	26	7	37		21	6	12
8	27	26	5	5	23	7	27
9	29	6	18		23	2	10
10	31	17	34	6	24	0	13
	Feb.				25	1	21
11	2	23	31	7	26	3	15
					27	9	14
				8	29	2	29
					29	5	23
				9	31	4	14
					31	7	19
					Feb.		
				10	2	9	14
					2	1	10
Total		100	254			67	346
Percentage of ♂♀		28.2				16.2	

Acidity

In one of Shull's ('11) earlier experiments it was found that sodium hydroxide reduced the proportion of male-producers to a slight extent. At the same time experiments with acids were performed in the hope of obtaining the opposite effect, but it was found impossible to rear the rotifers in even a very dilute solution of the inorganic acids used. Although weak solutions of hydrochloric acid were used, by the time the slightly alkaline food was added, the solution appeared neutral. When he used solutions of the acid strong enough to remain acid after the food was added, the rotifers died.

With the expectation that organic acids might be less deleterious, the following experiment with butyric acid was begun.

Experiment 2. A 1 per cent stock solution of butyric acid was kept in a glass stoppered bottle, and diluted for use to 0.03 per cent (the diluent being Great Bear spring water). Two lines of rotifers derived from two sisters isolated September 10, 1911, were reared, one in spring water, the other in butyric acid solution. For want of a satisfactory indicator, it was not known that the latter solution remained acid after the food was added. The characteristic odor of butyric acid remained, but it was to be expected that the butyrates would possess the same odor.

Contrary to our hopes, the acid reduced[2] the proportion of male-producers, as shown in table 2.

Delay or inhibition by manure solution

The non-occurrence of male-producers while the rotifers were being reared in strong manure solution might be attributed to delay, rather than to inhibition. So long as a line were reared continuously in manure solution the delay would be continuous; but if only one or two generations were reared in such a solution, might not male-production which could not occur in these

[2] It may be pointed out that the families are larger in butyric acid (mean size, 47.1 daughters) than in spring water (mean size, 38.7 daughters). According to Mitchell's conclusions ('13) based on Asplanchna but applied to rotifers in general, the higher nutrition evidenced by larger families should have been accompanied by greater male-production, instead of less.

TABLE 2

These two lines of Hydatina senta, derived from sisters, one line reared in spring water, the other in dilute butyric acid, show that butyric acid reduces the proportion of male-producers. It also increases the size of family

SPRING WATER				0.03 PER CENT BUTYRIC ACID			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	Sept.				*Sept.*		
1	12	0	46	1	12	3	51
2	14	4	19	2	15	0	42
3	16	6	23	3	17	0	52
4	17	0	26	4	18	0	45
5	19	0	28	5	20	2	46
6	20	7	45	6	22	1	45
7	22	11	44	7	23	4	39
8	23	7	44				
Total		35	275			10	320
Percentage of ♂♀		11.2				3.0	
Average size of family		38.7				47.1	

generations occur in greater degree in the generations immediately following them, which were reared in spring water? The possibility was tested in the following experiment.

Experiment 3. The general plan of the experiment was to rear, in spring water, females whose grandmothers had been kept in manure solution, and whose mothers were reared (except for a few hours after hatching) in spring water. The details of the method were as follows: Six or eight daughters of one female were divided into two lots of three or four each, one being put into spring water, the other into manure solution. Eight to 24 hours later these lots were transferred to new dishes, one in spring water as before, the other in manure solution. The transfer was made in order that the first young to hatch in the second dish of manure solution might be known to have hatched from eggs that underwent their maturation and part of their previous development in manure solution. These first young, if females, were transferred to spring water, in which (if they proved to be female-producers) they were kept the rest of their lives. Their entire families were reared to maturity and recorded. The totals for each lot of families simultaneously reared are shown in table 3.

TABLE 3

Records of male-producers and female-producers among females whose grandmothers were reared in manure solution, and females whose grandmothers were reared in spring water. All individuals other than these grandmothers, of all generations, and in both halves of the experiment, were reared in spring water. There is no accumulation of male-producers among granddaughters of females reared in manure solution

LOT NUMBER	GRANDDAUGHTERS OF FEMALES REARED IN SPRING WATER		LOT NUMBER	GRANDDAUGHTERS OF FEMALES REARED IN MANURE SOLUTION	
	Number of ♂ ♀	Number of ♀ ♀		Number of ♂ ♀	Number of ♀ ♀
A	9	44	A	32	37
B	4	51	B	1	38
C	2	40	C	4	70
D	33	44	D	30	37
E	9	13	E	19	19
F	28	16	F	15	83
Total......	85	208		101	284
Percentage of ♂ ♀...	29.0			26.2	

There is no increase in the proportion of male-producers among the granddaughters of females bred in manure solution. The figures show an actual decrease, but it is so small as to be probably insignificant. The manure solution does not merely delay, but inhibits, male-production.

EXPERIMENTS DESIGNED TO INCREASE MALE-PRODUCTION

In an attempt to increase male-production artificially a number of salts were tested, some of them being selected because of their well-known physiological effects in no way connected with sex, others selected purely at random. Only those in which the rotifers could be easily reared are reported in this paper. While this work was in progress Whitney's ('14) paper on nutrition experiments appeared. Our energy was then directed toward testing the agents which seemed to us to enter into Whitney's experiments but which he had apparently neglected. These two lines of work are discussed separately.

EFFECT OF VARIOUS SALTS

Experiment 4. Calcium chloride.[3] Two sister individuals of a line obtained in Nebraska were isolated on November 20 and 21, respectively, 1912. One, with its progeny, was reared in spring water, the other, with its progeny in $\frac{N}{100}$ solution of calcium chloride. Other conditions were the same in both lines. As shown in table 4, the only male-producers in this experiment appeared in the calcium chloride solution.

TABLE 4

The two lines of rotifers in this table were reared, one in spring water, the other in a dilute solution of calcium chloride. The only male-producers (♂ ♀) in all the families were produced in calcium chloride

SPRING WATER				$\frac{N}{100}$ CALCIUM CHLORIDE			
Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀	Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀
	Nov.				*Nov.*		
1	22	0	25	1	24	0	31
2	24	0	39	2	26	0	42
3	26	0	26	3	28	0	45
4	29	0	24	4	30	2	40
	Dec.				*Dec.*		
5	2	0	26	5	2	0	23
6	4	0	42	6	4	0	47
7	5	0	44	7	8	0	23
8	7	0	24	8	10	0	12
9	9	0	22	9	13	0	45
10	12	0	1	10	15	0	21
	14	0	9				
11	15	0	50				
Total		0	332			2	329
Percentage of ♂ ♀		0.0				0.6	

Experiment 5. More dilute calcium chloride. The above experiment was repeated, using a more dilute solution of calcium chloride. A $\frac{N}{300}$ solution was used in the first three generations, $\frac{N}{600}$ thereafter. In table 5, which records the details, it appears that three times as many male-producers were reared in calcium chloride as in spring water.

Experiment 6. Calcium chloride used intermittently. In this experiment, only one line was reared, beginning Jan. 11, 1913. Instead

[3] The results of these experiments with calcium chloride have been published in summary form elsewhere (Shull, '13) but appear here for the first time in detail.

TABLE 5

One of the two lines here recorded was reared in spring water, the other in a solution of calcium chloride more dilute than that used in table 4. Again there were more male-producers in the calcium chloride

SPRING WATER				$\frac{N}{300}$ TO $\frac{N}{600}$ CALCIUM CHLORIDE			
Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀	Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀
	Jan.				*Jan.*		
1	13	2	40	1	13	0	38
2	15	0	40	2	15	0	15
3	17	0	24	3	17	0	1
4	18	0	35	4	18	0	29
5	20	0	49	5	20	9	31
6	22	5	35	6	22	7	43
7	24	0	33	7	24	0	40
8	26	0	29	8	26	4	44
9	28	0*	7*	9	28	0*	11*
Totals		7	292			20	252
Percentage of ♂ ♀		2.3				7.3	

* Remainder of family not recorded.

of rearing it continuously in one medium, alternate generations were reared in spring water and a $\frac{N}{600}$ solution of calcium chloride. All members of one family were reared, from the time they were isolated shortly after hatching, in the same medium. That member of each family which was selected to become the parent of the next generation laid her eggs in the medium in which she was bred, and her eggs hatched there. But shortly after hatching, her daughters were transferred to the alternate medium, and there reached maturity.

Most of the male-producers appearing in this experiment, as shown in table 6, were offspring of females that lived and laid their eggs in calcium chloride solution. Since it is the growth and maturation period of an egg in which it is determined whether a male-producer or a female-producer will develop from that egg, as shown by the earlier experiments of Shull ('12), the results given in table 6 are what would be expected if calcium chloride favors male-production. In this respect Experiment 6 is in accord with the other calcium chloride experiments above.

TABLE 6

In the single line here recorded the first generation was reared in spring water (from a parent reared in $\frac{N}{400}$ calcium chloride); the second generation was reared in a calcium chloride solution (from a parent reared of course in spring water). Most of the male-producers are daughters of parents reared in calcium chloride

NUMBER OF GENERATION	DATE OF FIRST YOUNG	OFFSPRING OF PARENTS REARED IN SPRING WATER		OFFSPRING OF PARENTS REARED IN CALCIUM CHLORIDE	
		Number of ♂♀	Number of ♀♀	Number of ♂♀	Number of ♀♀
	Jan.				
1	13			0	26
2	15	0	14		
3	17			0	25
4	19	1	15		
5	21			4	41
6	23	0	23		
7	25			6	32
8	27	0	27		
Total		1	79	10	124
Percentage of ♂♀		1.2		7.4	

Experiment 7. Calcium chloride and a New Jersey line of rotifers. The effect of continuous rearing in calcium chloride of two concentrations was tested in a line of rotifers from New Jersey, to check the results in the three preceding experiments, in all of which the Nebrasla line was used. The method of conducting the experiment was the same, except that different concentrations were used, but, as may be seen from table 7, the results were different. There is a reduction in the number of male-producers in calcium chloride in this line.

We are unable to explain the difference between the Nebraska and the New Jersey line in this respect, though inherited physiological differences of other kinds are well known. The constancy of the results obtained with calcium chloride in the Nebraska line, even if the differences produced were small, lead us to attribute these effects with some confidence to the action of the calcium chloride.

Experiment 8. Magnesium chloride. The physiological effect of magnesium upon other organisms is in some ways in strong contrast to the effect of calcium. We hoped to find this difference extended to their effect upon the life cycle of Hydatina. A $\frac{N}{150}$ solution of magnesium chloride was used for one line, spring water in another

TABLE 7

Three lines of rotifers of a stock obtained in New Jersey were started from two sisters. One line was reared in spring water, the other two in two concentrations of calcium chloride. There are fewer male-producers in the calcium chloride solutions

Spring water			$\frac{N}{200}$ Calcium chloride			$\frac{N}{75}$ Calcium chloride		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	0	4	1	0	23	1	1	30
2	3	38	2	6	31	2	0	31
3	17	27	3	0	38	3	0	34
4	0	27	4	4	14	4	2	31
5	0	27	5	0	15	5	0*	15*
6	0*	11*	6	0*	10*	6	0*	13*
7	0*	14*	7	0*	11*	7	0*	8*
8	0	42	8	0	36	8	0	26
9	0	29	9	0	3	9	0	2
10	0	2		0	4			
	0	3	10	0	9			
Total	20	224		10	194		3	190
Percentage of ♂♀	8.1			4.8			1.5	

*Remainder of family not recorded.

line. Although both lines were reared for ten or more generations and over three hundred individuals were obtained in each line, not a single male-producer appeared in either line. It seems unnecessary to state such uniform results in detail, hence no table for this experiment is published.

There is no way of interpreting the results of this experiment. Magnesium chloride may reduce male-production. Or it may increase male-production, but could not overcome a strong 'tendency' to female-production in the line used. The experiment has never been repeated with a line in which male-producers appeared more frequently.

Experiments 9, 10, and 11. Potassium sulfate, iron chloride, and ammonium chloride. The success obtained by using very dilute calcium chloride suggested that very dilute solutions of other substances might also increase male-production, even in the case of substances,

more concentrated solutions of which were known to have the reverse effect. Among the substances tested were the three named above. Since all of them reduced male-production or showed no effect, the results of the several experiments are for brevity summarized in a single table (table 8).

TABLE 8

Summaries of several experiments with dilute solutions of potassium sulphate, iron chloride, and ammonium chloride. The number of male-producers is not increased by any of these substances, but in most cases is reduced

	EXPERIMENT					CONTROL			
Substance	Concentration	Number of generations	Number of ♂♀	Number of ♀♀	Per cent of ♂♀	Number of generations	Number of ♂♀	Number of ♀♀	Per cent of ♂♀
Potassium sulphate	$\frac{N}{200}$	8	19	343	5.2	8	31	326	8.6
Potassium sulphate	$\frac{N}{50}$	8	28	269	9.4	8	31	326	8.6
Iron chloride.......	$\frac{1}{600}\%$	5	8	158	4.8	5	43	157	21.5
Ammonium chloride..........	$\frac{1}{1000}\%$	9	0	241	0.0	10	3	356	0.8

FACTORS SUGGESTED BY WHITNEY'S EXPERIMENTS

Whitney ('14) reared Hydatina on a diet of Chlamydomonas, and obtained a striking increase in the proportion of male-producers. He freely attributed this effect to the (qualitative) difference in nutrition, without mentioning several agents whose possible effects had not been eliminated. For example, his food cultures were started in different media; Chlamydomonas was reared in a solution of bouillon cubes, the Polytoma used in the control was reared in manure solution. Although only small quantities of the liquids of the food cultures were introduced with the food, nevertheless there must have been some initial differences of a chemical nature between the experiment and its control. The possible effects of these substances were not tested, nor were they mentioned in Whitney's paper. Furthermore, it was probable that the organisms in the food cultures

produced, as a result of their metabolic activities, differences between the food cultures that did not exist at first. Thus, it might be expected that Chlamydomonas, a green organism, would carry on photosynthesis, with the resultant liberation of oxygen. It seemed not improbable that oxygen might produce part of the effects in the experiments with Chlamydomonas. This was the more plausible since, as Whitney states, marked effects were produced only when large numbers of Chlamydomonas were present, and only when the dishes were kept in direct sunlight. Both of the conditions named should result in the production of relatively large quantities of oxygen in solution.

If it should appear that any or all of these agents present in the Chalmydomonas cultures have any considerable effect in increasing male-production, Whitney's conclusion that his experiments gave evidence of an effect of nutrition would lose much of its support. If, on the other hand, these obvious factors could be shown to have no effect whatever, the belief that nutrition effected the increase of male-production noted would be greatly strengthened.

It may be stated in advance that our results may be interpreted as being largely in support of Whitney's contention; for, while one of the suspected agents (oxygen) gave positive results, its effect was much smaller than that which Whitney reported. Our experiments are the more significant because we have worked with a line of rotifers kindly sent to us by Dr. Whitney in January, 1915, from the line used in his own experiments. We can only regret that our experiments were not conducted by Dr. Whitney himself, as we have had some difficulty in duplicating his conditions merely from his published accounts.

In the account which follows, we separate the experiments aimed to test the effect of dilute bouillon from those designed to test the effect of oxygen.

Effect of dilute bouillon

Experiment 12. The bouillon solution used in this experiment was made from one cube of Armour's beef bouillon in 21,000 cc. of water. As nearly as we could compute from Whitney's paper, this was the concentration of bouillon present in his cultures after the rotifers had been fed with Chlamydomonas. Two lines of rotifers, derived from sisters, were reared, one in spring water, the other in this dilute bouillon solution. Table 9 shows a slight reduction in the number of male-producers in bouillon, rather than an increase.

TABLE 9

Showing the effects of continuous rearing of Hydatina in a dilute bouillon solution. The number of male-producers (♂♀) is slightly decreased

SPRING WATER			BOUILLON		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	0	15	1	0	11
2	6	17	2	3	32
3	2	19	3	0	3
4	0	17	4	0	17
5	0	29	5	0	8
6	1	24	6	1	29
7	0	14	7	0	20
			8	1	25
Total......	9	135		5	145
Percentage of ♂♀...	6.2			3.3	

Experiment 13. Bouillon was tested in this experiment by rearing only the parents in bouillon, not their offspring. This was Whitney's method in working with Chlamydomonas, and was based on Maupas's statement (1891), subsequently confirmed by Shull ('12), that the determination of the sex of an individual takes place in the body of its grandmother. The concentration of the bouillon was made less than that in Experiment 12, being one cube in quantities of water varying, on different days, from 30,000 to 80,000 cc. Two lots of females of approximately equal size and from the same source were set aside on the same day, the one in spring water, the other in bouillon solution. The parents were removed after 24 hours, The offspring from all eggs laid in that time were reared to maturity in spring water, and recorded. In table 10 the nature of these offspring is summarized. Here again there is a slight reduction in the number of male-producers in bouillon solution. The consistent results of these two experiments seemed to make it not worth while to look for a male-producing factor in the dilute bouillon solution used by Whitney.

TABLE 10

Showing the effects, on the offspring, of rearing the parents in dilute bouillon. The eggs from which the offspring hatched were laid and hatched in bouillon in one-half of the experiment, those of the other half laid and hatched in spring water. In both cases the offspring were removed to spring water a few hours after hatching

DATE	PARENTS REARED IN SPRING WATER			PARENTS REARED IN BOUILLON		
	Number of parents	Daughters proved to be		Number of parents	Daughters proved to be	
		♂♀	♀♀		♂♀	♀♀
January 27	3	2	10	4	0	11
30	4	0	59	4	2	42
February 5	3	0	39	4	0	65
9	3	0	28	3	0	27
16	3	18	20	3	9	38
22	3	0	54	3	0	52
March 1	2	3	7	3	0	8
Total	21	23	217	24	11	243
Percentage of ♂♀		9.5			4.3	

Effect of metabolic products of a green alga

In the hope of approximating the conditions of Whitney's experiments except the nutritive conditions, we reared the rotifers in water containing a green alga that the animals could not eat. Spirogyra was selected, though it was not known whether the metabolic products of Spirogyra are similar to those of Chlamydomonas or not.

Experiment 14. In this experiment, one of two lines derived from sisters was reared in water in which Spirogyra was kept, the other in water without Spirogyra. The same food was used for both. The Spirogyra was obtained from a spring, was kept in dishes in direct sunlight in the laboratory, and was washed out several times in Great Bear spring water before using, to prevent the introduction of foreign water in one part of the experiment. In the Spirogyra line only the parents and the first three or four daughters were kept in dishes with Spirogyra. The young females were removed daily to Great Bear water, in which they were reared to maturity. All dishes containing the parents, whether with Spirogyra or not, were in direct sunlight a part of the day. The temperature was not high when these experiments were performed (late winter and early spring), hence it was not necessary to take any precautions to reduce the temperature of dishes

TABLE 11

Showing the effect of rearing rotifers in the presence of Spirogyra. In one line the parents of each generation were reared in dishes containing Spirogyra; in the other line there was no Spirogyra in the parents' dishes. All offspring in both lines were reared in the absence of the alga

PARENTS REARED WITHOUT SPIROGYRA			PARENTS REARED WITH SPIROGYRA		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	8	42	1	14	41
2	11	42	2	12	28
3	1	38	3	2	18
4	2	18	4	7	43
5	3	22	5	6	36
Total.........	25	162		41	166
Percentage of ♂♀.....	13.3			19.8	

set in the sunlight. Bubbles, presumably of oxygen, were usually present in the Spirogyra dishes, indicating that photosynthesis was taking place.

As indicated in table 11, there is a noticeable increase in the number of male-producers in the Spirogyra cultures.

Experiment 15. The preceding experiment was repeated, this time with two lines in Spirogyra cultures, and but one control. The methods were the same as described for Experiment 14. Table 12 gives the results. In these lines there is practically no difference between the Spirogyra cultures and the control.

Experiment 16. Adopting for this experiment Whitney's method of rearing only the parents under experimental conditions, we used the following procedure. Two or three mature, egg-laying females, members of the same family, were placed in each of two dishes. In one was placed some Spirogyra, in the other none. Both dishes were put into a covered dish, which was floated on the water in a small aquarium, and kept in the sunlight at a south window during the major part of each day. A thermometer was put into the floating dish, and the temperature (at this time often quite high out of doors) was found to vary only two or three degrees during the day. After 24 hours the parents were removed. The eggs laid during this 24 hour period hatched where they were laid, and the young were removed to spring water to grow to maturity. These offspring are recorded in table 13. In this experiment there is a slight reduction in the number of male-producers, though it is so small as to be probably insignificant.

TABLE 12

Showing the effects of continuous rearing of the rotifers in water containing Spirogyra, as in Table 11. Two lines were reared in Spirogyra cultures, with a single control

WITHOUT SPIROGYRA			WITH SPIROGYRA			WITH SPIROGYRA		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	1	10	1	0	8	1	0	32
2	1	30	2	1	19	2	8	16
3	2	10	3	0	33	3	0	16
4	3	32	4	0	19	4	1	23
5	1	9	5	3	21	5	4	51
6	3	42	6	2	20	6	0	19
7	0	13	7	0	13	7	0	3
			8	0	10			
			9	6	37			
Total..........	11	146		12	180		13	160
Percentage of ♂♀..........	7.0			6.2			7.5	

TABLE 13

Recording the offspring of parents reared in Spirogyra cultures, as contrasted with those of parents reared in the absence of Spirogyra. All offspring were reared in spring water. Spirogyra used in this way does not increase male-production. See also tables 11 and 12

WITHOUT SPIROGYRA			WITH SPIROGYRA		
Number of parents	Daughters		Number of parents	Daughters	
	Number of ♂♀	Number of ♀♀		Number of ♂♀	Number of ♀♀
3	3	15	2	0	14
3	5	18	3	2	22
2	2	40	2	7	35
2	7	28	2	4	22
2	1	19	2	1	18
Total.........	18	120		14	111
Percentage of ♂♀........	13.0			11.2	

On the basis of the Spirogyra experiments as a whole, we are unable to draw a definite conclusion. While by continuous rearing with Spirogyra an increase in the number of male-producers was one time obtained, at another time the increase was practically zero; while when only the parents were reared in Spirogyra cultures, the offspring were slightly less frequently male-producers than in the control.

Effect of oxygen

The effect of oxygen was directly tested in several experiments. The method, in general, was to put the rotifers into a dish of water previously saturated with a mixture of air and oxygen containing a known proportion of oxygen, then set the dish in an atmosphere containing the same proportion of oxygen. The two atmospheres used were composed, respectively, of 40 per cent and 60 per cent oxygen. The oxygen was obtained by heating potassium chlorate with manganese dioxide and collecting the gas in a gas holder over water. No attempt was made to purify the oxygen.

The 40 per cent oxygen atmosphere for saturating the water was obtained by lowering a graduated tube, fitted at one end with a stop-cock and open at the other end, into a vessel of water, admitting first three volumes of air, and then one volume of oxygen from the gas holder. The mixture was subsequently driven into a flask of spring water inverted over water, and later shaken repeatedly with the water remaining in the flask. When the rotifers were put into a dish of this oxygenated water, the dish was set under a bell jar made air-tight at the bottom with a vaseline-coated rubber gasket. By means of a filter pump, one-fourth of the air under the bell jar was removed, as indicated by a mercury manometer in connection with the bell jar. Oxygen from the gas holder was then admitted until the mercury fell to zero. The partial exhaustion and refilling of the bell jar occupied usually less than two minutes. The 60 per cent oxygen atmosphere was obtained in the same way, except that equal parts of air and oxygen were mixed.

Experiment 17. Continuous rearing in 40 per cent oxygen. Two sisters were isolated March 31, 1915, one in spring water, the other in similar water first saturated with an atmosphere composed of 40 per cent of oxygen. The former dish was kept in air, the latter under a bell jar in an atmosphere containing 40 per cent of oxygen. From each parent was reared a line under the same conditions as those in which the parent female was placed. In the oxygen line, only the parents and several additional members of each family were kept in oxygenated water. Four or five of the first daughters of each family were kept in oxygenated water, and from among them the parent of the next generation was selected. The remaining daughters in each family were transferred to spring water, where they reached maturity. Whatever effect the oxygen has, therefore, must be exerted in early larval stages, or in the egg, or in the body of the mother. From the previous experiments of Shull ('12) it is to be expected that the egg or oögonial stages are the only ones in which the life cycle can be altered.

TABLE 14

Recording two lines derived from sisters, one reared in untreated spring water, the other in water which was first saturated with an atmosphere of which 40 per cent was oxygen, then placed in a corresponding atmosphere. The oxygen line yields more male-producers than the control

AIR				40 PER CENT OXYGEN			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	Apr.				*Apr.*		
1	3	0	16	1	3	2	28
2	6	1	36	2	4	1	27
	6	0	29	3	6	2	32
3	8	0	8	4	8	4	46
	9	0	6	5	10	7	43
4	10	3	48	6	12	4	42
5	12	11	43	7	14	10	39
6	14	8	33	8	16	2	39
7	16	1	22	9	18	0	8
8	18	0	6		19	0	12
	19	0	8	10	20	0	14
9	20	0	23	11	22	12	38
10	22	4	23		22	4	35
11	23	1	35	12	23	2	44
12	25	1	35	13	25	9	25
Total		30	371			59	482
Percentage of ♂♀		7.4				10.9	

Table 14 records the results of this experiment. There is a small increase in male-production in the oxygen line.

Another feature of the oxygen line that is perhaps of significance in connection with the next experiment, is the distribution of the male-producers in the family. In any line of Hydatina the male-producers tend to occur in groups; that is, several successive members of a family will be male-producers. This grouping is most apparent in lines in which male-producers are abundant; for, though the most frequent number of male-producers in a group is always one, by far the majority of male-producers in such a line occur in much larger groups. When the proportion of male-producers is 30 to 40 per cent, it is not uncommon to find groups of ten, twenty, or even thirty or more male-producers occupying successive places in the family. The cause of this grouping is unknown, but evidently when the conditions are right for the appearance of male-producers, they remain so for a time, then disappear or are inoperative.

Were there no such grouping, in a line yielding only 5 or 10 per cent of male-producers, most of these would necessarily occur in groups of one. Groups of two would be very uncommon, while larger groups would be almost unknown.

It is interesting, therefore, especially in view of the fact that large groups are quite common in lines in which male-producers are abundant, to compare the distribution of the male-producers in the two lines of table 14, one having a higher proportion of male-producers than the other. The daily records of these two lines have been examined, and the number of times that one, two, three, four, or five successive members of the family were male-producers was recorded. There were no groups larger than five. If such records are to be trustworthy, it is essential that the order of individuals in the family be known. There is a possibility of error here, for the order of age was determined each day by the order of size. In the young females the differences in size usually left little doubt as to their relative ages, but errors were undoubtedly sometimes made. However, errors were no more probable in one line than in the other; and there could have been no bias, either conscious or uncon-

scious, on our part, since the order was determined long before it was known which ones would be male-producers.

The number of groups of successive daughters, of one to five members, comprising only male-producers, is summarized in table 15. The slight increase in the mean number of male-producers per group in the oxygen line is not in proportion to

TABLE 15

Showing the number of times that groups of one, two, three, four, or five successive members of the families in table 14, were male-producers

NUMBER OF MALE-PRODUCERS OCCURRING IN SUCCESSION	NUMBER OF GROUPS IN AIR LINE	NUMBER OF GROUPS IN OXYGEN LINE
1	15	27
2	4	10
3	1	1
4	1	1
5	0	1
Mean size of group.......	1.42	1.47

the increase in the total number of male-producers in that line. The additional male-producers called forth by the oxygen appear, therefore, in additional groups of small size, rather than as additional members of the groups which would otherwise appear. Thus, there is an increase in the number of groups, rather than an increase in the size of the groups. This means a more uniform distribution of the male-producers through their respective families in the oxygen line.

Experiment 18. Continuous rearing in 60 per cent oxygen. This is a repetition of Experiment 17, but with an atmosphere of which 60 per cent was oxygen, instead of 40 per cent. The methods used were otherwise the same. Table 16 gives the results by generations. There is no increase in the number of male-producers in the oxygen line; whether the slight decrease is significant is not known.

Although there is practically the same proportion of male-producers in both lines in this experiment, the distribution of these male-producers over the families is more uniform in the oxygen line than in the control. This is shown first by the number of male-producers occupying successive places in the

TABLE 16

Showing number of male-producers (♂♀) and female-producers (♀♀) in two lines of Hydatina, one reared in water in ordinary air, the other in water saturated with an atmosphere of which 60 per cent was oxygen

AIR				60 PER CENT OXYGEN			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	June				*June*		
1	1	1	20	1	1	4	31
2	3	0	3	2	3	0	9
	4	0	1	3	5	2	22
	6	3	13		5	4	25
3	7	12	10	4	7	3	37
4	9	10	31	5	9	4	17
5	11	0	10	6	11	1	3
6	14	0	5	7	14	5	28
	14	0	1	8	17	16	16
	14	2	28	9	19	3	42
7	15	13	23	10	20	1	8
8	17	23	20		21	11	38
9	19	3	26	11	22	19	31
10	21	15	23	12	25	7	31
11	23	1	46	13	27	11	23
12	25	15	36	14	28	14	25
13	27	18	34	15	30	2	25
14	29	4	45		*July*		
15	30	6	32	16	2	6	11
	July			17	4	1	30
16	3	24	24	18	6	35	12
17	4	9	39				
18	6	24	29				
Total............		183	499			149	464
Percentage of ♂♀		26.8				24.3	

families. Table 17 records all these groups of successive male-producers in both lines. The average size of these groups in the air line is 3.26, in the oxygen line only 2.40. The male-producers are distributed more uniformly through the families in the oxygen line than in the control. In this connection, see also table 15, and the discussion of it under Experiment 17.

This difference in the distribution of the male-producers is also seen if, instead of recording the generations separately, as

TABLE 17

Showing the number of groups (of various sizes) of successive male-producers in the families of the two lines recorded in table 16

NUMBER OF MALE-PRODUCERS OCCURRING IN SUCCESSION	NUMBER OF GROUPS IN AIR LINE	NUMBER OF GROUPS IN OXYGEN LINE
1	32	33
2	4	18
3	4	2
4	3	3
5	5	2
6	2	1
7	0	2
9	3	0
12	1	0
19	1	0
24	1	0
32	0	1
Mean size of group of successive male-producers	3.26	2.40

in table 16, one examines the daily output. In each day's product occur members of several successive generations. As Shull (1915) has pointed out, rhythmical production of male-producers may often be more easily detected by an examination of daily records than by a study of family records. In table 18 is recorded the number of male-producers and female-producers on each day in each line of table 16, regardless of the families to which they belong. The percentages of male-producers are computed for tri-daily periods, in order to smooth some of the enormous fluctuations in both lines. These tri-daily percentages are graphically shown in figure 1, where it is seen that the oxygen line is plainly more uniform than the control line.

If it be objected that the daily records should not be combined for comparison, that combination may be rejected, and the difference between the two lines is still plainly visible. The number of male-producers appearing on successive days in the control line is subject to greater extremes of fluctuation than in the oxygen line. Of the 39 days through which the experiment extended, there were twelve days on which no male-producers ap-

TABLE 18

Showing the number of male-producers and female-producers appearing each day in the families recorded in table 16, regardless of the families to which they belonged

DATE	AIR LINE			60 PER CENT OXYGEN LINE		
	Number of ♂♀	Number of ♀♀	Per cent of ♂♀	Number of ♂♀	Number of ♀♀	Per cent of ♂♀
June						
1	0	2		0	1	
2	0	5	5.8	1	11	10.7
3	1	9		2	13	
4	0	0		0	6	
5	0	8	15.7	1	11	12.5
6	3	8		4	18	
7	1	8		1	23	
8	4	4	50.0	1	16	10.7
9	11	4		5	19	
10	6	7		2	6	
11	0	13	15.0	0	10	14.2
12	0	14		1	2	
13	0	6		0	1	
14	0	6	0.0	0	7	0.0
15	0	6		0	6	
16	2	18		2	3	
17	0	17	22.2	5	10	37.9
18	10	7		4	5	
19	7	13		7	19	
20	10	12	44.2	5	16	17.6
21	10	9		0	21	
22	3	22		2	15	
23	6	13	26.2	7	22	27.1
24	7	10		10	14	
25	1	21		4	17	
26	1	23	10.2	5	13	28.5
27	6	26		9	15	
28	9	21		4	20	
29	17	13	29.5	9	15	22.0
30	3	35		4	25	
July						
1	2	29		6	21	
2	0	11	11.9	4	7	25.0
3	6	19		2	8	
4	5	11		2	7	
5	14	11	44.0	5	6	26.6
6	14	20		1	9	
7	10	16		9	18	
8	14	15	38.7	13	6	56.6
9	0	7		12	2	

peared in the control line, only seven such days in the oxygen line. On the other hand, there were nine days on which the control line yielded ten or more male-producers, only three such days in the oxygen line. The second of the above-mentioned differences may be partly due to the greater absolute number of male-producers in the control line; but if allowance be made for this fact, the former difference becomes all the more striking.

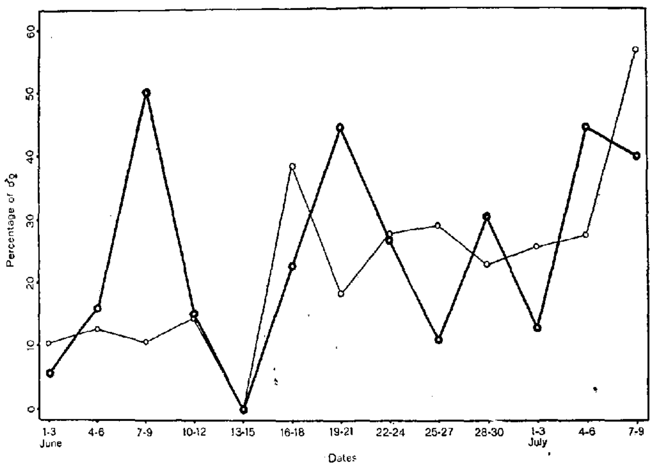

Fig. 1 Graphic representation of the proportion of male-producers, computed for three-day periods, in the two lines recorded in tables 16 and 18. The light curve represents the oxygen line, the heavy curve the control. The fluctuation is less in the oxygen line.

A further difference between the two lines is the existence of four fairly distinct waves of male-production in the control line, each separated from the others by periods of few male-producers. Such a rhythm in the oxygen line is less distinct, or in part wanting.

These differences are all due to a more uniform distribution of the male-producers over the families of the oxygen line, and over the period of the experiment in the oxygen line, than in the control. Thus, although the oxygen did not cause an increase in the number of male-producers, it was not without its effect upon male-production.

Why the 60 per cent oxygen in this experiment did not increase male-production, whereas the 40 per cent oxygen of the preceding experiment did, is not known. Different concentrations of the same agent may have different effects. Or oxygen may increase male-production only when the other conditions present are rather unfavorable to male-production. That the other conditions were right for high male-production is shown by the fact that the control line produced 26.8 per cent of male producers, as against 7.4 per cent in Experiment 17. The stock of rotifers used was, at the time of the experiment, in one of its waves of high male-production. This may be the reason why the oxygen could not still further increase the number of male-producers.

Experiment 19. Oxygen counteracting bouillon. This and the following three experiments were suggested by the possibility, mentioned in the preceding paragraph, that oxygen could increase male-production only when other conditions were rather unfavorable to male-production. The several experiments immediately following show the influence of oxygen in counteracting the effects of agents known to reduce the number of male-producers.

In this experiment, a one-seventh per cent solution of Armour's beef bouillon cubes was used. Only the parents were reared in the bouillon, not the offspring (this being Whitney's method). The parents were put into bouillon for 10 to 14 hours, then transferred to a new dish of bouillon. This transfer was made to insure that all the eggs laid in the second dish went through their maturation stages in the bouillon. The bouillon in the second dish was first saturated with an atmosphere containing 40 per cent oxygen, and the dish was then placed under a bell jar, in a 40 per cent oxygen atmosphere. The parents remained in the second dish for twenty-four hours, at the end of which time they were removed. All the eggs laid in the second dish of bouillon were allowed to hatch there, after which the young were transferred to spring water, where they grew to maturity.

The control parents were kept only in spring water which was kept in ordinary air. Every time the parents in bouillon were transferred to a new dish, the control parents were likewise transferred to fresh spring water, to prevent the unequal accumulation of metabolic products in the two lines.

Table 19 shows the results of the experiments. The oxygen appears not only to have counteracted the effect of the bouil-

lon, but to have added a substantial balance of male-producers on the bouillon side.

TABLE 19

Showing the effect of oxygen and bouillon on one line, as contrasted with a line not subjected to either. Bouillon, even in dilute solutions, has been shown to reduce the number of male-producers (see table 9). In this experiment, oxygen counteracts the effect of the bouillon, and actually increases the proportion of male-producers above that in the control line

SPRING WATER, WITHOUT OXYGEN			BOUILLON, WITH OXYGEN		
Number of experiment	Number of ♂♀	Number of ♀♀	Number of experiment	Number of ♂♀	Number of ♀♀
A	3	23	A	1	24
B	1	11	B	10	4
C	1	23	C	0	8
D	0	14	D	0	3
E	0	16	E	0	23
F	0	26	F	0	12
G	0	13	G	0	9
H	1	22	H	6	12
I	0	3	I	0	11
J	0	22	J	14	20
K	0	6	K	0	2
Total............	6	179		31	128
Percentage of ♂♀	3.2			19.5	

Experiment 20. Oxygen counteracting strong manure solution. In this experiment only the parents were reared in the manure solution, the offspring being in all cases reared in spring water. The parents to be used for both experiment and control were placed in manure solution in the afternoon; the next morning one lot (the control) was transferred to a new dish of manure solution, the other lot was transferred to manure solution which had been saturated with an atmosphere of which 40 per cent was oxygen. The former lot was kept in air, the latter under a bell jar in an atmosphere of 40 per cent oxygen. At the end of 24 hours the parents were removed from both dishes. All eggs laid in the 24 hour period were allowed to hatch where laid, the young females being then removed to spring water.

The young females from each lot of parents were transferred to spring water on two successive days. When the parents were removed, those eggs that were laid in the first seven to ten

hours had already hatched. These females were transferred to spring water at that time; in table 20 they are recorded as 'older daughters.' On the following day all the remaining eggs

TABLE 20

The parents were reared in strong manure solution, but in one-half of the experiment this solution was saturated with an atmosphere 40 per cent of which was oxygen. All offspring were reared in spring water. The oxygen approximately doubled the proportion of male-producers

DATE	MANURE SOLUTION, AIR					MANURE SOLUTION, 40 PER CENT OXYGEN				
	Number of parents	Older daughters		Younger daughters		Number of parents	Older daughters		Younger daughters	
		♂♀	♀♀	♂♀	♀♀		♂♀	♀♀	♂♀	♀♀
Apr.										
28	5	0	17	0	12	4	0	13	0	13
29	3	1	1	3	17	3	0	4	0	8
30	3	0	7	0	9	3	0	4	0	1
30	2	0	2	0	6	2	1	5	0	5
May										
4	2	0	0	0	15	2	0	4	1	12
4	2	0	3	0	13	2	0	4	2	11
5	2	0	5	0	17	2	0	3	0	6
5	2	0	5	0	24	2	0	9	0	17
6	2	0	5	1	13	3	0	18	0	15
6	4	0	12	1	37	3	0	17	0	19
7	2	0	6	0	14	3	0	11	0	18
7	4	0	9	0	29	3	0	9	0	16
9	4	0	19	2	48	4	0	17	3	33
10	3	1	8	0	26	4	0	25	1	38
12	3	0	22	0	23	2	2	16	1	18
14	3	0	6	0	25	3	0	8	1	16
16	3	0	11	3	17	2	0	7	1	11
18	1	0	2	1	8	3	0	4	2	5
20	4	2	8	0	7	4	2	13	0	22
22	3	0	14	0	7	2	0	7	3	10
24	4	3	16	2	22	4	6	23	6	23
26	3	0	1	0	17	3	1	5	0	21
28	5	0	13	0	50	5	3	21	6	33
30	3	0	2	0	20	3	2	4	0	10
Total..	72	7	194	13	476	71	17	251	27	381
Percentage of ♂♀		3.4		2.6			6.3		6.6	

had hatched; the young females were removed to spring water, and appear in table 20 as 'younger daughters.' The sex-ratio for the older and younger daughters is given separately to show that the effect of the oxygen is as marked at first as it is later. The proportion of male-producers is approximately doubled by the oxygen.

Experiment 21. Oxygen counteracting weak manure solution. In this experiment two lines were bred continuously in weak manure solution. From the first several daughters of each generation one was selected to become the parent of the next generation. One line was reared in a dilute manure solution saturated with a 40 per cent oxygen atmosphere, the other line in a similar (but not oxygenated) solution. In the former line, only the parents and the first few daughters of each family were kept in the oxygenated solution, the other daughters being transferred to spring water. The method of conducting the

TABLE 21

Two lines of rotifers, both reared in dilute manure solution, are here recorded. One line was kept in manure solution saturated with an atmosphere of which 40 per cent was oxygen. The oxygen increased the number of male-producers

DILUTE MANURE SOLUTION, AIR				DILUTE MANURE SOLUTION, 40 PER CENT OXYGEN			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	July				*July*		
1	9	1	40	1	9	3	14
2	11	0	42	2	11	0	33
3	13	1	18	3	13	0	33
4	14	0	21	4	14	13	14
5	16	0	31	5	16	1	14
6	17	0	6	6	17	0*	3*
7	19	3	40		18	0	11
8	20	5	19	7	19	1	40
9	22	2	45	8	21	13	37
10	24	2	32	9	22	2	33
11	25	0	31	10	24	4	40
12	27	0*	23*	11	25	5	25
				12	27	1*	25*
Total		14	348			43	322
Percentage of ♂♀		3.8				11.7	

* Remainder of family not recorded.

experiment was the same as in Experiment 17, except that a manure solution was used instead of spring water. Table 21 shows that the oxygen increased the number of male-producers.

Experiment 22. Oxygen counteracting creatin. In the experiments of Shull ('11) it was shown that creatin was one of the most effective agents in the reduction of the number of male-producers. A more dilute solution of creatin was used in the following experiment. The parents alone were reared in the creatin solution. On one side of the experiment this solution was saturated with an atmosphere of which 40 per cent was oxygen. The parents on both sides were kept only 24 hours. All eggs laid in that time were allowed to hatch, and the young were reared to maturity.

Table 22, which records this experiment, shows that oxygen increased the number of male-producers.

TABLE 22

The parents of the rotifers here recorded were reared in solutions of creatin of varying concentrations; but in one-half of the experiment, the creatin solution was saturated with an atmosphere of which 40 per cent was oxygen. All offspring were reared in spring water. The oxygen increased the number of male-producers

DATE	STRENGTH OF CREATIN, IN PER CENT	CREATIN, AIR			CREATIN, 40 PER CENT OXYGEN		
		Number of parents	Number of ♂♀	Number of ♀♀	Number of parents	Number of ♂♀	Number of ♀♀
Dec.							
7	0.01	3	1	21	3	2	20
8	0.01	3	0	7	3	0	8
9	0.005	3	1	24	3	6	27
11	0.005	3	13	10	3	12	16
12	0.005	3	9	8	3	25	1
13	0.005	3	1	15	3	12	17
14	0.005	2	2	13	3	7	10
16	0.005	4	6	19	4	19	15
18	0.0083	3	0	16	3	1	10
19	0.005	4	1	15	4	2	22
20	0.0083	4	3	12	4	0	9
21	0.0083	5	1	12	5	3	22
Total..............		40	38	172	41	89	177
Percentage of ♂♀..........			18.1			33.4	

DISCUSSION

The more recent investigations upon Hydatina, conducted chiefly by Whitney and Shull, left no doubt as to the main problem which those investigations were designed to solve. External and internal factors *both* determine the amount of male-production. But the evidence of the operation of external factors was so abundant that new problems were at once created. All such agents at first discovered, and they were not few in number, had the same effect; they reduced male-production. It became important to discover the methods, or preferably method, by which these very diverse agents produced their common result; for by that means it appeared most likely that the solution of the second new problem would be reached. This latter problem was to discover a method of increasing male-production. Only in this way, it seemed, was it likely that the ultimate aim of these studies, the discovery of the physiological phenomena accompanying changes in the mode of reproduction, would be attained.

The former question, namely, that regarding the modus operandi of various chemical substances in retarding male-production, is still unanswered. The experiments described in the first pages of this paper, on osmotic pressure, acidity, and possible after effects of manure solution, led us to no conclusions. Failure to solve this problem has made the attack of the second problem, discovery of means of increasing male-production, largely a matter of trial and error. Even by this method some success has been attained; experiments 4, 5, and 6 show that calcium chloride has the desired effect upon certain lines of rotifers. Whitney's fortunate discovery that feeding the rotifers on a green flagellate increased male-production gave us our only clew. We have discussed Whitney's experiments above on pages 137 to 139, and will not repeat here.

Repeated experiments with practically uniform results have demonstrated, to our satisfaction at least, that part of the increased male-production following the use of Chlamydomonas as food, in Whitney's cultures, was due to the oxygen liberated

by the green flagellates as a by-product of photosynthesis. Our results show marked effects of oxygen. However, the increase of male-production with oxygen alone is by no means as great as Whitney obtained with Chlamydomonas. We suspect, therefore, that although all the factors obviously associated with Chlamydomonas in the cultures should be separately tested before any residue of influence is assigned to nutrition, Whitney's conclusion that the food conditions influence male-production is correct, though that influence is less than he believed. We are convinced, nevertheless, that *this influence of nutrition is dependent chiefly, if not wholly, upon quality, not quantity, of food.* Although large quantities of Chlamydomonas in Whitney's cultures produced more marked results than small quantities, this is to be attributed, we think, to the greater quantity of oxygen evolved. It can not be assumed that any 'law of mass action' holds for the ingestion of food by organisms. That is, after a certain optimum (probably moderate) quantity is reached, further 'concentration' of the food does not necessarily increase the nutrition of the devouring animal. But such concentration of a green flagellate does increase the concentration of oxygen in solution. If experiments with moderate quantities of Chlamydomonas show an increase of male-production, it is probable that such increase more nearly represents the effect of nutrition itself.

With the discovery that oxygen increases male-production are we any nearer a knowledge of the fundamental causes of changes in the life cycle? We are inclined to answer in the affirmative. It may be recalled that male-production is ordinarily subject to marked periodicity (Mitchell, '13; Shull, '15). Periods of many male-producers are also usually periods of rapid growth and reproduction. Lines that yield many male-producers are also usually vigorous. Metabolic processes are going on at a rapid rate. It is hard to avoid the suspicion that in some way this speed of reaction in the protoplasm is related to the production of males. Probably not all metabolic processes, but only certain ones, are thus related to the sex-ratio. For, while, as just stated, periods of male-production are usually

also periods of rapid growth, not all periods of rapid growth are accompanied by many male-producers. And while lines producing many males are usually vigorous lines, there are equally vigorous lines (judged by all known standards) that produce comparatively few males. These facts may be harmonized with the view that speed of reaction within the protoplasm makes for male-production, if we assume that only certain reactions bear this relation to sex.

If we adopt the view that the rate at which certain chemical events proceed determines the form of the life cycle, the positive results of our present experiments have some meaning. The dilute solution of calcium chloride merely provides a medium that is slightly more favorable to the processes concerned. Oxygen obviously provides for accelerated oxidation in the protoplasm. The effect of oxygen aside from male-production is not merely inferred; for the rotifers in oxygenated water, and those in Spirogyra cultures, were almost invariably healthier than the control. Sometimes the families in the oxygenated water were larger, but always the animals were more easily reared to maturity. If qualitative differences of nutrition affect the sex-ratio, as we must on the basis of Whitney's experiments assume that they do, these may likewise be conceived to affect the speed of the metabolic reactions. Even quantitative differences of nutrition, should these eventually be found to alter the sex-ratio, could conceivably alter the metabolic processes; indeed, there are no a priori grounds for believing that they do not.

The suggestion that mere speed of reaction is responsible for varying degrees of male-production becomes more plausible if its operation can be visualized in terms of known processes. It is well established, we believe, by the investigations of Shull ('12, pp. 302–308), that male-production is either caused or prevented at some time within the growth and maturation period of every parthenogenetic egg. When an egg has passed its maturation stages, the fate of the female which will hatch from that egg is sealed. She will be either a male-producer or a female-producer, according as one or another series of events

has taken place in growth or maturation, and her nature is no longer subject to alteration. What happens to decide this fate is unknown. It may be the failure of some chromosome to divide. The male-producing female may have fewer chromosomes than the female-producer. The apparent variability of the number of chromosomes (Whitney, '09) may be due, not entirely to difficulty in counting them, but partly to actual differences.

If such behavior of the chromosomes results in the development of a male-producer, the rate of formation of the spindle, or of the division of chromosomes, may be the cause of the chromosome change. A chromosome dividing a little later than its fellows may be drawn (?) to one pole without completing its division. The cytology of the germ cells of Hydatina should be re-examined, with a view to discovering the difference between male-producing and female-producing females. But we emphasize that our theory of the speed of reaction is not bound up with chromosomes, to stand or fall with future discoveries regarding the chromosomes of these rotifers. Other phenomena than chromosomes which have, with present technique, no visible expression, may as conceivably be influenced by the speed of metabolic processes.

If male-production is related to the rapidity with which certain physiological processes occur, it is not surprising that a change of environment more often reduces male-production than increases it. A high degree of male-production, on our view, depends upon an efficient mechanism working at top speed. Any unskilled workman may ruin a delicate machine, it takes an inventor to improve it. Many changes of environment may retard metabolic processes, only a few accelerate them. We should expect, therefore, that most agents which affect the life cycle at all would reduce male-production, and this has been the case.

It may be suggested that our view is very near that of Whitney ('14), Mitchell ('13), and Nussbaum ('97), that nutrition is the controlling factor in the life cycle of Hydatina. If nutrition be re-defined to include all chemical processes in proto-

plasm, the suggested factor may be classed under nutrition. But by so defining nutrition the term metabolism becomes superfluous. Furthermore, it is clear from the context that none of the writers just named meant anything more by nutrition than quantity or quality of food devoured. We care not about the terminology, the ideas are distinct.

SUMMARY

The common effect of numerous substances upon the life cycle of Hydatina senta (diminution of male-production) is not due to their osmotic pressure, acidity or alkalinity, nor to mere delay of certain processes.

Calcium chloride, in very dilute solutions, repeatedly increased male-production in one parthenogenetic line, not in another. Magnesium chloride gave results that could not be interpreted; while potassium sulfate, iron chloride, and ammonium chloride all reduced male-production. Dilute bouillon also diminished male-production.

Oxygen in the water increases male-production. Its effect is most marked in the counteraction of agencies which diminish male-production, such as bouillon, manure solution, and creatin. Whitney's experiments with Chalamydomonas, in which male-production was greatly increased, an effect which he attributed to nutrition, are partly explained, therefore, as dependent upon the oxygen evolved in photosynthesis. Our results, however, were not as marked as Whitney's, and experiments in which the rotifers were reared with a green alga too large to be eaten, gave negative results. It is probable that nutrition has some effect, as Whitney supposed, but to what extent can not be known until the other agents which can not be eliminated are separately tested.

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A LIST OF CORRECTIONS

Crozier, W. J. The rhythmic pulsation of the cloaca of holothurians. Jour. Exp. Zoöl., vol. 20, pp. 297–356, April, 1916.

p. 299, line 18 from top: for "Mr." read "Dr."
p. 305, line 5 from bottom: "oral" should read "anal."
p. 310, line 3 from bottom: for "smoother" read "smooth."
p. 315, the figure on this page lacks *number* and *legend;* the inscription "Duration of pulsation in minutes" is misleading; it should read: "Time subsequent to amputation, minutes."
p. 316, line 2 from bottom: for "table 2" read "table 1."
p. 320, line 2 from bottom: for "convoluta" read "Convoluta."
p. 321, line 8 from bottom: after "intact half" insert "(fig. 20)."
p. 323, line 5 from top: for "(fig. 20)" read "(fig. 21)."
p. 323, line 7 from top: at end of line insert "(fig. 20)."
p. 324, line 8 from top: delete "(fig. 21)."
p. 328, line 2 from bottom: for "variability" read "viability."
p. 332, table 8, line 6: sign "=" should be "±."
p. 333: figure 25 lacks its *number* and *legend.*
p. 335: in legend of figure 27, second line, "5 per cent urea" should read "$\frac{M}{10}$ urea."
p. 348: figure 30 lacks *number.*
p. 352: footnote 20, line 1: for "arrangements" read "arrangement."
p. 306, line 3 from bottom; p. 334, line 3 from top; p. 336, line 3 from bottom; p. 338, line 4 from top; p. 343, line 5 from top; p. 346, line 6 from top; and p. 354, line 10 from bottom: for "1915" read "1915 [?]."
Note—The paper referred to was in type late in 1914; but it is not known when it was issued, if at all.

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CHEMICAL CONTROL OF RHEOTAXIS IN ASELLUS

W. C. ALLEE

Marine Biological Laboratory and Lake Forest College[1]

TEN FIGURES

CONTENTS

I. EARLIER WORK AND METHODS

Certain conditions known to affect the metabolism of animals are known regularly to affect the rheotactic reactions of the isopod Asellus communis Say. Lowered oxygen tension, chloretone, potassium cyanide, lowered temperature, suddenly increased temperature, increased carbon dioxide tension, and

[1] The experiments upon which this paper is based were carried on at Woods Hole during the summers of 1913, '14, and '15. During this time 1003 individual isopods were tested in a total of 13,245 minute reaction periods. I am indebted directly and indirectly to many people with whom I have talked during the progress of the work, particularly to Dr. F. B. Coffin, for aid with certain phases of the chemistry of the problem.

THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, VOL. 21, NO. 2
AUGUST, 1916

starvation, all of which depress the rate of animal metabolism, also decrease the positive rheotactic response of these isopods. Heightened oxygen tension, caffein, and a gradual increase in temperature have the opposite effect ('12, '13).[2]

Changes in the percentage of the positive rheotactic reaction are correlated with resistance to potassium cyanide ('14) and with changes in carbon dioxide production (Allee and Tashiro '14). At the present time their resistance to cyanide and their carbon dioxide production furnish the best known indices of the rate at which metabolism is carried on in these small organisms.

Essentially the same method of testing the rheotactic tendency has been used throughout this series of isopod experiments. The animals to be tested, usually three in number, are placed in a waxbottomed enamel ware pan 25 cm. in diameter and 6 cm. deep in 2 cm. of water to which they are accustomed. The pan is placed in diffuse light and after standing for about fifteen minutes a current is set up by stirring with the bulb end of an ordinary pipette. Rheotactic reactions given in a circular current have been found comparable with those given in a straight current and because of the greater speed with which tests may be made the circular current method has been exclusively used in these experiments. Great care is taken to create an approximately uniform rate of current. The reaction of each isopod is taken for one minute and these are separately recorded. As a rule this is repeated ten times but with highly toxic chemicals the preliminary interval was shortened and only five trials were made.

The chemicals used were Kahlbaum's c. f. a. in all cases except caesium chloride which was Baker's 'Standard Purity.' The solutions were made with distilled water using the molecular weights in Chemiker Kalender for 1913. Allowance was made for water of crystallization but no quantitative tests were run even in the deliquescent salts. However, the experiments on calcium chloride and magnesium chloride were repeated each

[2] Numerals standing alone refer to my earlier papers.

of the three summers that the work was in progress with similar results so that weight variations must have been slight.

The isopods used in these experiments were all Asellus communis Say, collected from the 'dump pond' in Woods Hole. The animals used were mainly immature and were rarely in the laboratory more than three days before experimentation began. When first collected they gave the low rate of positive reactions that is usual for Asellus from such ponds. They were kept in the laboratory under low or high oxygen conditions according to the demands of the experiments for animals with low or high rates of positive rheotaxis.

II. ELECTROLYTES

Chlorine salts of the alkali metals

All the chemicals tested in the concentrations used will cause a decrease in the positive rheotactic reaction if allowed to act for sufficient time; and at one stage the investigation virtually became a search for reagents that would cause an increase in the positiveness of the reaction. Of the cations tested only rubidium and potassium showed a strong consistent increase. Sodium and barium have similar but less pronounced effects.

The results of the trials with the chlorine salts of the alkaline metals in N/10 solutions (except sodium chloride which is N/5) are given in figure 1. The cations are arranged in order of their atomic weights which are shown by the solid line with short cross marks. Each space in the ordinates equals 5 units of the atomic weight. As the atomic weight of these metals increases the ionic velocity and electronegativeness decreases. The simple broken line gives the percentage that at some time in the treatment showed some increase in the positiveness of their rheotactic reaction. The dotdash line shows the difference in the percentage of positive rheotaxis before treatment and at the most positive period during treatment. In both these lines each space in the ordinates has a value of 5 per cent.

The unbroken line in the figure represents the relative toxicity of the different salts which is reckoned by the average time in

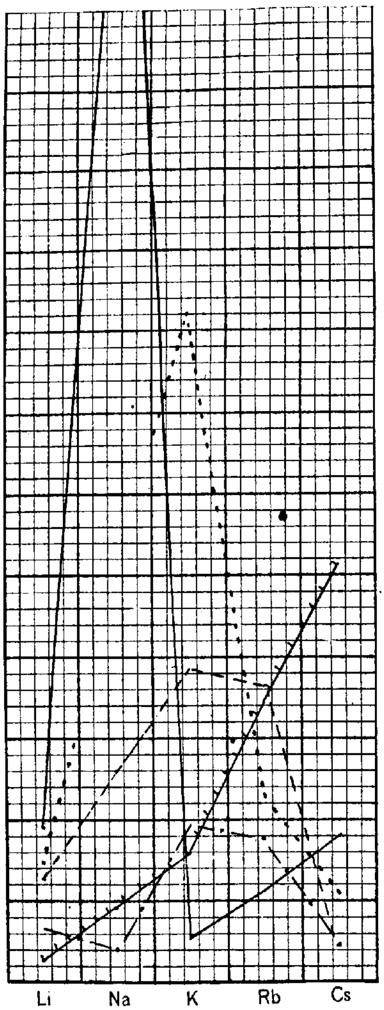

Fig. 1 Showing relative toxicity and relative effect of chlorine salts of the alkali metals on positive rheotaxis of Asellus. For methods of charting see text p. 165.

minutes elapsing before the isopods lost the power to stand. This turning time may or may not be correlated with the survival time of the individual but since it shows the time during which the animal may react it is more important in the study of certain phases of behavior than the death time of the animal. In this curve each space in the ordinates represents twelve minutes. The dotted line gives the ratio existing between the beginning of depression and the turning time reduced to percentage of the latter. This ratio will be called the toxicity. depression ratio and is shown here with each ordinate space worth 2 per cent.

The distribution of the experiments on which these curves are based is shown in table 1.

TABLE 1

Showing the number of rheotactic experiments performed with the different strengths of the chlorine salts of the alkali metals

CATION	N/5	N/10	N/20	N/40
Li	17	24		
Na	78			
K	2	74	7	
Rb		89		
Cs		21	9	27

If one considers the percentage of isopods having their rheotactic reaction made more positive the order of effectiveness is Li < Na < K > Rb > Cs. When the amount of increase in positiveness is compared N/5 sodium chloride is less effective than N/10 lithium chloride but if N/5 solutions of both salts are compared the former is more effective and the above order holds for the amount of increase as well as for the number made more positive. If this series be written in the ordinary physiological style it is Cs < Li < Na < Rb < K with a sharp division between the sodium and rubidium. This reminds one of the usual physiological order of these cations except that caesium which usually lies between lithium and sodium in its effects on living tissues and colloids (Höber '14, s. 487) here is less effective than either.

The chlorides of potassium and rubidium were found to be most efficient in increasing the positiveness of the rheotactic reaction. These salts are chemically closely related and must represent the optimum of the series of conditions that are found in the alkali metal series. The effect of these two cations (with the chlorine anion) is so striking that it is worth while exhibiting the result of their action. The effect of rubidium chloride may be seen in table 2 and that of potassium chloride in table 5, p. 190.

TABLE 2

Showing some diagrammatic reversals in the sign of the rheotactic reaction caused by N/10 RbCl

ISOPOD NO.	RHEOTAXIS BEFORE TREATMENT				RHEOTAXIS AFTER TREATMENT				TIME TRE'T'D	SIZE IN MM.	TEMP.
	+	−	α	E[1]	+	−	α	E[1]	min.		
514		100		2.25	100			1.0	16	7.5	25
525	30		70	0.90	80	20		1.0	10	6.0	21
526		100		2.65	60	20	20	1.7	16	7.0	21
527		100		3.25	80	20		0.5	16	8.0	21
528		90	10	1.65	60	20	20	0.8	8	6.0	23
529		100		3.00	100			1.7	17	6.0	23
531	10	90		2.55	100			1.8	26	6.0	23
532	20	20	60	1.00	100			1.4	15	7.0	23
533	10		90	1.10	80		20	1.2	15	7.0	23
Ave...	8	67	25	2.04	84	9	7	1.2	15	6.7	

[1] The figures in these columns give in a standardized from the amount of movement of the isopods during their rheotactic test. For detailed account see '13, p. 261.

The cases listed in the rubidium table are selected and are the most diagrammatic obtained with that reagent while those shown for potassium include all the tests for a given experiment which explains the apparently greater efficiency of rubidium.

The stimulating effect of potassium and rubidium chlorides was noticeable in the general behavior and particularly in the increased rapidity of movement of the isopods. One of many similar laboratory notes for rubidium (N/10 solution) is: "Isopod became nervously active immediately although it had been sluggish before." Another for potassium (N/10) is "All nervous, excited, although losing power of coördination." Continued

exposure even to these stimulating salts results in a decrease in the positiveness of the reaction but at times, particularly in potassium chloride, this decrease comes only when the isopods lose coördination through the toxic action of the solution. They may either lose power of positive orientation while still strongly stimulated or may orient repeatedly but be unable to hold the position. While in this state the isopods have a tendency to run in small circles of about a centimeter in diameter. Similar circular reactions have been observed under natural conditions but not to the extent produced in these solutions. Sodium chloride, the next most stimulating salt, gives none of these phenomena and its stimulating action is slower as well as less pronounced.

It has been shown that a relationship exists between the positiveness and the efficiency (the distance covered during a minute reaction period) of the rheotactic reaction ('13). This has since been amply confirmed in the study of reactions under natural conditions or under such depression as may be caused by calcium chloride or cane sugar. That the two phases of the reaction are not correlated at all times is shown by the experiments with rubidium and potassium chlorides just mentioned and is typically illustrated by the results shown in table 2. In the trials listed there the positiveness of the isopods was increased markedly in all cases but in all save one the efficiency of the response decreased, and while the positive rheotactic reaction was increased 76 per cent the efficiency was cut almost in half. In the very beginning of the treatment the activity increased as has been noted but the subsequent depression often came before the isopods lost the power of positive orientation.

This toxicity-depression ratio (dotted line figure 1) which was high in the case of potassium and relatively so with rubidium was quite low with lithium and caesium both of which depress the rheotactic positiveness long before the toxic effect is apparent. The ratio was not found for sodium because the toxicity was not accurately determined.

In considering the relative toxicity of the different alkali metal cations it should again be noted that the comparison in the figure (unbroken line) is between N/5 sodium as compared

with N/10 solutions of the other cations. Even with this difference sodium is least toxic, followed after a long interval by lithium, caesium, rubidium and potassium in the order named. The favorable effect of sodium chloride is shown by the fact that ten isopods were reacting after eleven hours and at fourteen hours six of these were more positive than at the beginning. Three were still reacting after sixteen hours and lived for five days in N/5 solution.

The cation NH_4 is chemically closely related to the other members of this group and is often similar in physiological action to potassium which it particularly resembles. In its effect on rheotaxis, however, ammonium chloride is less effective than caesium chloride so that the complete series would be $NH_4 < Cs < Li < Na < Rb < K$. The toxicity effect of ammonium is like that of potassium and rubidium as shown by the series $Na < Li < Cs < Rb < NH_4 < K$. This is in the same order as has been found to preserve the irritability of fresh frog's nerve (Brodsky '08, vide Höber s. 511); to cause recovery of irritability after loss in cane sugar solution (Höber, s. 497) namely: $Na < Li < Cs < NH_4 < Rb < K$. The only difference is that in the foregoing instance ammonium stands next to rubidium while with the isopods it is next to potassium.

I am unable to state why ammonium should act in its usual manner as regards toxicity and not as regards rheotaxis. The rheotactic reaction may be influenced by the well developed nervous system of the isopods or by direct action on the muscles or by a combination of the two. It may be we have here a differential effect in that potassium stimulates the muscles more than it depresses the central nervous system while ammonium acts in the opposite manner. Mathews ('07) found that these two cations have similar effect on muscles and on motor nerves but are opposed in their effect on the central nervous system, which potassium depresses while ammonium stimulates it to the point of tetanic convulsions. The action may also be explained by assuming that ammonium produces a greater permeability than potassium such as Lillie ('09) discovered with Arenicola larvae. It is known (Mathews '07) that the am-

monium salts may be eccentric in their action and Mathews interprets this as due largely to their hydrolytic dissociation but this explanation is unsatisfactory so far as the present work is concerned because of the slight amount of this kind of dissociation in the solution strengths used.

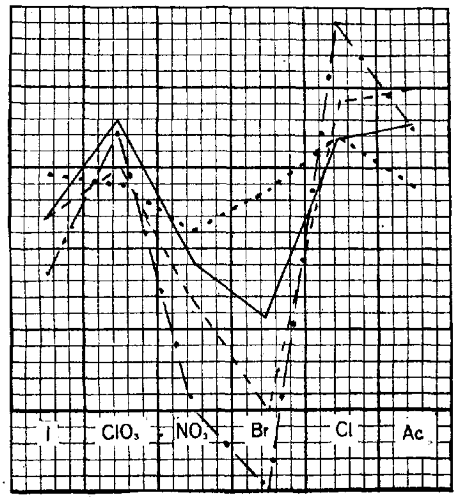

Fig. 2 Showing relative toxicity and the relative effect of different anions with the cation potassium on positive rheotaxis of Asellus. For details see text p. 172.

Different anions with the cation potassium

Some work was carried on to find whether different anions with the most effective cation, potassium, also affected rheotaxis. The studies were only carried far enough to demonstrate that the anions are effective and to suggest in a tentative way the order of their relative effect. The findings with N/10 solutions, which was the strength most often used, are shown in figure 2.

Figure 2 is charted in much the same manner as the preceding figure. The anions are arranged in their usual relative colloidal

and physiological effect (Höber, s. 308). Again the simple broken line shows the percentage of isopods that were made more positive at some time during the treatment with each ordinate space worth 5 per cent. The dot-dash line gives the average difference between the percentage of positive rheotaxis before and at the most positive point after treatment with each ordinate space equal to 2 per cent. The unbroken line gives toxicity, each ordinate space representing one minute, and the dotted line shows the toxicity-depression ratio with the ordinate spaces worth 5 per cent.

Experiments with potassium sulphocyanide in N/5 solution showed that it was more toxic than any other anion tried at this strength. In N/20 solution it caused some increase in positive rheotaxis but the evidence of the experiments at hand is contradictory and there are too few instances to locate this anion definitely. Potassium sulphate (2N/7 and N/15) increased the positiveness of the response, especially in the weak strength where it was almost as effective as potassium chloride in N/10 solution but more toxic.

As the figure shows the relative toxicity was $Br > NO_3 > I > Cl > Ac$, ClO_3, and the effect on positive rheotaxis was $Br < NO_3 < I < ClO_3 < Cl < Ac$ if one considers the effectiveness in causing some increase in the positiveness. The same order holds true when the extent of the increase is considered except that *Ac* is less effective than ClO_3 and Cl. I am not prepared to state why the order found here differs from the usual relative effect shown by the order of arrangement in the figure or why the more toxic salts should vary in their toxicity and their effect on rheotaxis. It is possible that more experiments might change the order in one or two places, but enough tests have been made to demonstrate conclusively that potassium chloride is the most effective salt so far as amount of change is concerned, and the regularity with which bromine takes the usual place of iodine at this concentration indicates that the order found is not a mere accident. A number of the anions that failed to show a marked increase in N/10 solution were tested in N/20 and N/40 concentration. No marked change occurred except in

the bromide-iodide relation just mentioned. At the weakest concentration the bromide became more effective than either the nitrate or the iodide and took its usual place in the anion series viz: $NO_3 < I < Br < Ac$.

It is noteworthy that the toxicity-depression ratio is uniformly high which means that the potassium cation, regardless of the anion, tends to cause the isopods to give a positive reaction well after the amount of activity is markedly depressed. The variation in effect of these anions on rheotaxis must be due in part to some other factor than relative toxicity because the toxicity-depression ratio does not vary sufficiently to account for the rheotactic results obtained.

Chlorine salts of the alkali earths

Although any chemical at the concentrations used will ultimately cause a decrease in rheotactic positiveness, calcium and strontium chlorides were found to be particularly effective in causing this depression without preliminary stimulation. Barium and magnesium chlorides also cause an ultimate depression but they often give a preliminary period of stimulation.

Some of the experimental data on which these conclusions are based are shown in table 3 which shows the toxicity of M/5

TABLE 3

Showing the toxicity and effect on rheotaxis of chlorine salts of alkali earths. Strontium chloride experiments were run in N/5 and the others in M/5 solutions. Isopods with an initial response of more than 71 per cent positive were not considered

TOXICITY				RHEOTAXIS		
Cation (1)	Number (2)	Toxicity time (3)	Depression / toxicity (4)	Number (5)	Per cent more positive (6)	Amount of increase (7)
Mg	10	8 hrs.[1]	21	32	31	−9
Ca	6	3 hrs.[1]	6	21	0	−36
Sr	11	58 min.	26	7	14	−11
Ba	12	1 hr. 30 min.	36	10	50	+18

[1] Active after.

solutions of barium, calcium and magnesium chlorides and of N/5 solution of strontium chloride, together with their effect on positive rheotaxis. The different columns have the following significance: No. 2 shows the number of isopods on which the toxicity time was determined; No. 3 gives the average toxicity; No. 4 gives the ratio of toxicity-depression in percentages of the toxicity time; No. 5 lists the number of rheotactic tests made whose results are shown in the next two columns; No. 6 shows the percentage of isopods that showed some increase in rheotactic positiveness during the treatment and the last column gives the amount of the increase or decrease at the most positive period of treatment.

Strontium chloride is decidedly more toxic than any of the others; barium comes next and after an interval comes calcium with magnesium much the least toxic. Calcium chloride at M/5 concentration failed to give a preliminary increase in the positive rheotaxis but it did cause an increase in 5 per cent of all the trials made at all concentrations. Calcium also caused a marked decrease in positiveness even when the most positive responses are considered. The low percentage shown by the ratio of depression to toxicity indicates that unlike potassium and rubidium the depression is not due primarily to toxicity.

Strontium chloride acts much as calcium chloride except that it is less effective in the concentration used and more toxic. Although magnesium stimulated approximately one-third of the isopods during the first 45 minutes of treatment the average effect of the treatment was a decrease in positiveness.

Barium chloride caused a greater initial stimulation than any other chloride tried excepting only those of potassium and rhubidium and possibly sodium. In percentage of individual stimulated, in amount of stimulation, and in the relatively high toxicity-depression ratio barium aligns itself with the alkali metal group. This is in keeping with its usual physiological action which has been known since Ringer ('86) found that it acted with the alkali metals rather than as the other alkali earths.

Antagonisms

When isopods are treated alternately with potassium and calcium chlorides the well known antagonistic action of the two salts is plainly shown. The effect of such treatment on eight isopods is graphically exhibited in figures 3, 4, and 5. In making these trials the isopods were tested as usual in water to which they were accustomed, then dried momentarily on filter paper and introduced into the desired solution. After about a minute they were given five one minute trials and again placed in water.

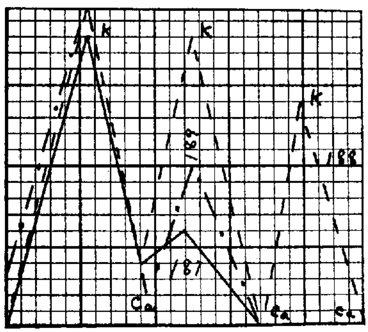

Fig. 3 Showing antagonistic action of N/10 potassium chloride and M/5 calcium chloride on positive rheotaxis of three isopods. For explanation see text.

After about two minutes they were placed on filter paper and introduced into the other solution. Experiments showed that this handling had a stimulating effect but this was not sufficient to mask either the antagonistic effects here recorded or the depressing effects of most salts tried.

The method of charting is the same in all three figures. One space in the ordinates is worth 5 per cent, one in the abscissae equals five minutes. The lines show the variation in the percentage of positive responses given and in all cases at the beginning of the treatment, the low points mark the response when treated with calcium, the high ones when treated with potassium chloride. The only exceptions are found in figure

5 after the isopods had been treated with alternating solutions for over two hours. The reactions of the different individuals are shown by the different lines and are duly labeled in the figures. No. 188 is repeated in figure 4 for the sake of comparison. Figures 3 and 4 are from work done in 1913 with N/10 potassium against M/5 calcium chloride and figure 5 is from 1914 results with both solutions 1/10 normal.

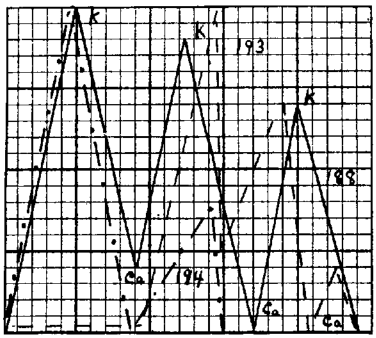

Fig. 4 Showing antagonistic action of N/10 potassium chloride and M/5 calcium chloride on positive rheotaxis of three isopods. For explanation see text p. 175.

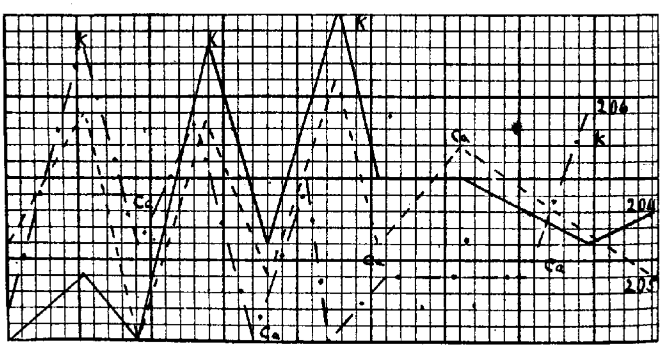

Fig. 5 Showing antagonistic action of N/10 solutions of potassium and calcium chlorides on positive rheotaxis of three isopods. For explanation see p. 175.

Tests were made for similar antagonisms between sodium and magnesium chlorides which are less effective in influencing the rheotactic response than the ones just given. No evidence of marked antagonism was found until the isopods were left in the different solutions for several hours when a definite antagonism was exhibited as is recorded in figure 6 where each space in the abscissae represents an hour.

Acids (H ions)

It is particularly hard to summarize in brief form detailed results of the experiments with acids. Preliminary tests were run with acetic, N/100, hydrochloric; N/50, N/100, N/500

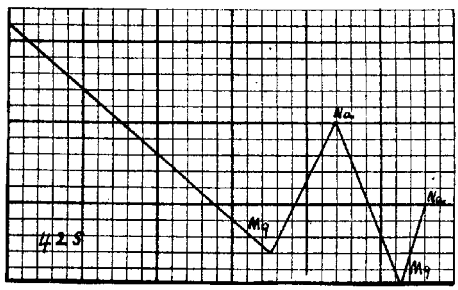

Fig. 6 Showing antagonistic action of N/5 sodium and magnesium chlorides on positive rheotaxis of one isopod.

and N/1000; sulphuric, N/100, N/500, and N/1000; and with nitric acid N/100. There was some slight evidence of stimulation with N/1000 hydrochloric and with N/100 acetic acids. These reagents accordingly were tried again the following season and 3 isopods with N/100 acetic acid showed complete depression while 15 trials with 6 isopods using N/1000 solution of the same acid showed depression in all cases save one. A similar repetition with N/1000 hydrochloric showed only one stimulation.

To test the matter further a fairly complete series was run with N/4000 hydrochloric acid the results of which are shown in figure 7. In this series 36 trials were made with 14 isopods

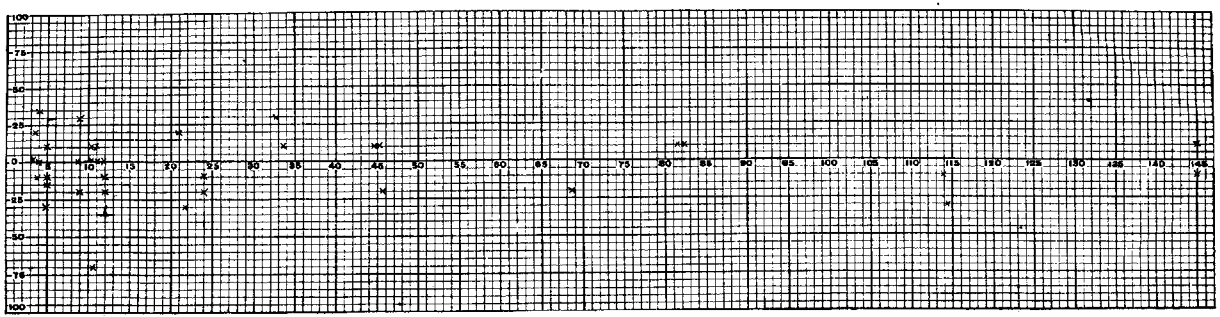

Fig. 7 Showing the effect of N/4000 hydrochloric acid on positive rheotaxis. Each space on the abscissae represents an hour and on the ordinates, 5 per cent change in the positive rheotactic reaction. Further details on p. 177.

whose initial response was under 71 per cent positive with one exception where the reaction was 90 per cent positive at the start. This individual was made more positive by the action of the acid and so may fairly be included (p. 178). Each space in the abscissae represents one hour and in the ordinates 5 per cent change in the positive rheotaxis. The location of the cross gives then the increase or decrease in the positive rheotaxis and the time that the isopod had been subjected to the treatment. The crosses on the base line indicate in this experiment that neither before nor after the treatment did the isopods give a positive response.

In brief the results show that 14 tests of 5 isopods showed an increased positiveness averaging 15 per cent, 6 tests showed no change in the positive reaction though considering the change in negative, indefinite and zero responses each indicated a depression; and that in 16 tests 9 isopods showed a decrease averaging 21 per cent. As a final summary 39 per cent of the trials showed some increase but the average amount of change in the positive reaction was a decrease of 13 per cent. This is similar to the results obtained with magnesium chloride and indicates a depression.

In the more concentrated solutions acids are decidedly toxic as is shown by the following records for hydrochloric acid:

Solution strength	*Time before all are on back*
N/5	1 minute
N/20	8 minutes
N/50	120 minutes
N/100 Out of three tried two were dead in 24 hours	

The toxicity was greater than with any other chlorine compound tried and by the usual reasoning in such cases this must be ascribed to the hydrogen ion and the depressing action of acids on rheotaxis must also be ascribed to this ion because each anion tried had the opposite effect on rheotaxis when combined with some other cation. By this I do not mean to maintain that the action of the cation is necessarily direct but that the cation here is the important part of the combination either through its direct action or because it fails to entirely

neutralize the effect of the anion. As to which of these possibilities is correct my work offers no direct proof.

The depressing effect of acids on nervous tissue is well known to physiologists (Mathews '04). They antagonize sodium chloride (Osterhaut '14) but less than calcium chloride and they depress permeability as also does calcium. The effect on rheotaxis is in accord with this work but with it opposes the observations of Bohn ('12) that N/1000 sulphuric acid increases the oxidation rate of lobster larvae and thereby causes them to become positive to light, and those of Lillie ('09) that N/400 and weaker solutions of hydrochloric acid increase permeability and stimulate Arenicola larvae.

Alkalies (OH ions)

Mathews ('04) found that hydroxyl ions are most efficient nerve stimulants. Bohn ('12) found that lobster larvae were made positive to light by N/1000 sodium hydroxide and states that the action is similar to that of sulphuric acid but less rapid. Loeb and Wasteneys ('13 a) report that sodium hydroxide has no effect on the rate of oxidations of fertilized Strongylocentrotus eggs unless the concentration is above N/1000 and ('15) that this minimal concentration varies with different species. More concentrated solutions cause a marked increase in the oxygen consumption but the eggs are injured. Osterhaut ('14) found that in the above strength sodium hydroxide makes practically no change in the permeability of Laminaria. Kanda, ('14) using N/20 potassium and sodium hydroxides obtained no reversals in geotaxis with Arenicola larvae although in common with Bohn and earlier workers he did obtain reversals in the reaction to light. Lillie ('09) using N/25–N/100 sodium hydroxide found no stimulation of Arenicola larvae and suggests that it decreases permeability.

Potassium and sodium hydroxides were tested for their effect on rheotaxis, the former in N/50 and N/200 solutions and the latter in N/200 and N/500. With the weaker solution of potassium hydroxide two of six isopods were slightly stimulated and

the average rheotactic reaction was depressed by 25–37 minutes treatment from 40, 32, 28 to 23, 25, 52 per cent positive, negative and indefinite. The isopods lost correlation in this solution in about two hours making the toxicity-depression ratio approximately 31 per cent which is the lowest ratio shown by any potassium salt. None of the six isopods tested with sodium hydroxide were made more positive and the average rheotactic reaction was also markedly depressed.

These results with alkalies obviously are based on too little work to conclude that hydroxyl ions will not stimulate positive rheotaxis but since there was no evidence of such stimulation the tests were discontinued.

Distilled water

To what extent are the results just recorded due to osmosis? It will be remembered that Asellus is a fresh water isopod inhabiting in this case pond water that must exert some osmotic pressure, especially during the summer season when the solutes present are more concentrated. Obviously however the effects recorded cannot all be due to osmosis because as has been shown equimolecular solutions of certain of the salts had opposite effects. For example N/10 potassium chloride with an osmotic pressure of about four atmospheres is very efficient in causing isopods to give a more positive response. The same strength solution of calcium chloride with an osmotic pressure only slightly greater has the opposite effect. In all probability these were more concentrated than the pond water to which the isopods were accustomed.

By the use of distilled water it is possible to obtain a condition where the osmotic pressure is less than that of the pond water though the action of distilled water may be due to some other factor than osmosis. Water redistilled in glass is quite toxic for these isopods but the water furnished by automatic laboratory stills will allow the isopods to live as long as five days. Bullot ('04) obtained similar results with the fresh water Gammarus.

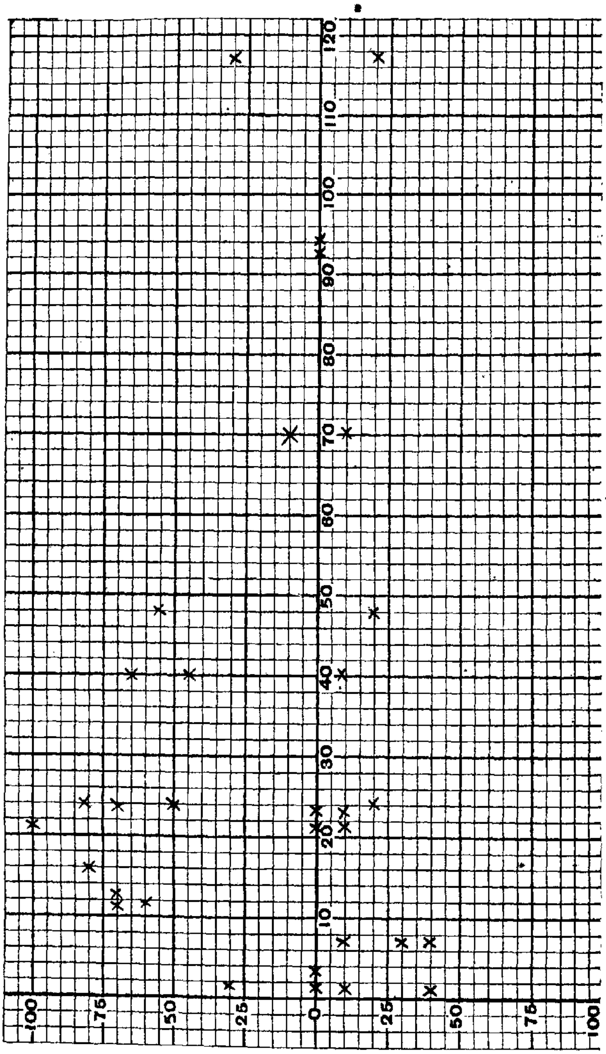

Fig. 8. Showing the effect of distilled water on positive rheotaxis. Each space on the abscissae represents two hours and on the ordinates 5 per cent change in positive rheotaxis. *Further details on p.* 183.

The results of experiments with 11 isopods are shown in figure 8. The charting is the same as in the preceding figure except that the abscissae spaces represent two hours in place of one. As usual, responses obtained from isopods with an initial reaction of over 70 per cent positive are disregarded. Of the isopods tested 55 per cent were stimulated at some time during their treatment. The 17 of the 35 tests which showed some increase in the positive response averaged 49 per cent more positive than before treatment. Six showed no change in the positive reaction and the 12 trials that were less positive gave an average decrease of 24 per cent. Altogether there was an average increase in positiveness of 16 per cent for the 35 trials.

Peters ('04) found that 'very pure' distilled water causes Stentor to lose salts but that the animals do not swell, i.e., there is no intake of water. Garrey (vide Mathews '04) reports that treating Chilomonas with distilled water increased their irritability and Mathews suggests this is because the protoplasm is brought more nearly to the neutrality point. However it is possible that there is an intake of water and there is some evidence (Riddle '14) that such an increase would result in an increased metabolic rate which could account for the increase in positive rheotaxis. This subject will be considered further on p. 186.

III. NON-ELECTROLYTES

Cane sugar

Cane sugar (rock crystal sugar) in M/2 solution exerts an osmotic pressure of about 12 atmospheres (see references to work of Morse and his students in literature list)[3] which is near three times that exerted by N/10 potassium or calcium chlorides and if the depression or stimulation results given by these salts were due to osmosis cane sugar should give greatly increased effects. That the sugar at this strength causes depression is shown in table 4, which exhibits results obtained by exposing 24 isopods to M/2 cane sugar for 52–75 minutes. As usual

[3] Garrey '15 found that 1/2 G. M. cane sugar solution gave −1.15 to −1.155 which would give an osmotic pressure of about 14 atmospheres.

the reactions of isopods with a high initial positive response are not considered.

The averages show that from a rheotactic reaction of 13, 73, 12, 2 per cent respectively positive, negative, indefinite and zero the isopods were changed to 11, 19, 63, 7. The decrease in positiveness is insignificant but the decrease in the negative reaction and the accompanying increase in indefinite responses shows depression in animals with a low initial positive reaction

TABLE 4

Showing the effect of M/2 cane sugar solution on rheotaxis of isopods with an initial response of less than 71 per cent positive. In all cases reported the treatment was from 52 to 75 minutes.

I indicates an increase and D a decrease in positive rheotaxis caused by the treatment

	BEFORE TREATMENT						AFTER TREATMENT					
No.	+	−	α	O	E.	Size	+	−	α	O	E.	Effect
237		20	80		1.1	6.5		50	40	10	1.0	I
239	70	10	20		1.8	7.0			80	20	0.7	D
244		90	10		1.7	6.0	20		80		1.4	I
245	10	80	10		1.7	6.5		40	50	10	1.2	D
246		50	20	30	0.9	7.0		10	90		0.6	D
247	70	10	20		1.4	6.0	30	20	50		0.9	D
248	40	30	20	10	1.5	7.0	20	30	50		0.9	D
249		90	10		2.4	8.0		10	80	10	0.7	D
260		100			2.2	7.0		20	80		1.0	D
261		100			2.0	6.5		30	10	60	1.2	I
263	30	70			0.7	8.0			40	60	0.7	D
265		100			2.3	6.0	30	30	40		1.6	I
266		90	10		2.1	7.0			60	40	0.4	D
267	10	90			2.3	7.5			70	30	0.7	D
283	10	90			2.1	5.0			100		0.7	D
285		100			2.3	7.0			100		0.6	D
280		100			1.2	5.0			60	40	0.4	D
281		100			1.8	6.5	20	10	60	10	1.3	I
282		10	90		2.0	7.5	30	40	30		1.6	I
277	50	50			2.0	7.0			70	30	1.1	D
278		100			2.5	8.0	50	10	40		1.2	I
286	10	90			1.6	6.0			100		0.6	D
287		100			1.6	6.0	10	50	40		1.0	D
288	10	80	10		1.9	7.0	20	10	70		0.8	I
Ave...	13	73	12	2	1.8	6.5	11	19	63	7	0.9	

just as a decrease in positiveness does when isopods are highly positive at the start. Nine isopods or 38 per cent of those tested were stimulated by this treatment for this length of time. The isopods reacted to water currents after fourteen hours exposure to sugar solutions and fully recovered from the depression when placed in tap water. Needless to say the longer treatment caused a greater depression.

Although cane sugar depresses positive rheotaxis it does not do so to the extent that would be expected if the effects of the calcium ions reported above were due to osmosis. No experiments were run testing whether or not the effects of sugar could be offset by different ions as in muscle or nerve preparations but three attempts at recovery using distilled water were somewhat successful. The best case follows: The rheotactic reaction of isopod 290 was changed by three hours treatment with M/2 cane sugar from 80 per cent positive, 20 per cent indefinite to 20 per cent negative, 60 per cent indefinite, 20 per cent zero. After 3 hours 30 minutes in once distilled water the response was 60 per cent positive, 40 per cent indefinite. This is not the expected result if distilled water acts by a differential removal of salts for cane sugar should act in the same manner. Loeb ('03) found with a marine Gammarus that distilled water and cane sugar solutions had approximately the same toxic effect and ascribed this to the loss of electrolytes or ions into each solution. He suggested that the exit of antagonistic salts takes place with unequal rapidity or in unequal relations. If the effect on rheotaxis were explained on this basis one would have to assume that calcium or strontium salts escape into distilled water and potassium or sodium salts into cane sugar and finally that by withdrawing first enough of one set and then enough of the other the rheotactic reaction would be restored to its original condition. This may be what happens but the observed results can be amply and more simply explained on the basis of a change in water content, with the distilled water allowing an increase in water which should increase the metabolic rate as suggested above and the cane sugar removing water which should decrease the rate of metabolism, and this is what actually happens under treatment with cane sugar (p. 193).

IV. METABOLISM AND RHEOTAXIS

Child ('15 and citations) has established a relationship between the metabolic rate of many lower animals and plants and their resistance to potassium cyanide. Hyman ('16, 16a) obtained similar results for certain annelids and sponges and I have found that this relationship holds for Asellus ('14). Geppert ('89) for certain mammals and birds, Warburg ('10), Loeb and Lewis ('02), Loeb ('06) and Loeb and Wasteneys ('10, '13) for sea urchin eggs found that the addition of cyanide decreased markedly the oxygen consumption; Hyman ('16 a) gives more detailed results of this relationship with sponges. It appears more than probable that the cyanide acts by affecting the oxidations but as Child ('14) says:

> Susceptibility to cyanide in concentrations which are lethal within a few hours varies with the general rate of metabolic activity or of certain fundamental metabolic reactions. This conclusion holds whether the cyanide acts more or less directly upon oxidations or upon the condition of the metabolic substratum or certain of its constituents and so indirectly upon metabolism in general.

Does resistance to sodium cyanide measure the metabolic rate of Asellus?

Certain observations that isopods much depressed by treatment with calcium chloride became more active after being put into N/1000 potassium cyanide and that isopods from potassium chloride did not, led to a fear that the cation even in this dilution might have some effect on the death point, particularly with isopods previously treated with either of the above salts. For this reason it was thought desirable to substitute sodium cyanide with its less toxic and less stimulating cation. The expectation was not entirely realized for animals treated with calcium chloride until they had lost power to move regained it slightly in sodium cyanide. It is possible that the cyanide itself causes a slight initial stimulation (Loevenhart '06), Hyman ('16 a).

The use of sodium in place of potassium cyanide required a retesting to determine what strength, if any, would measure

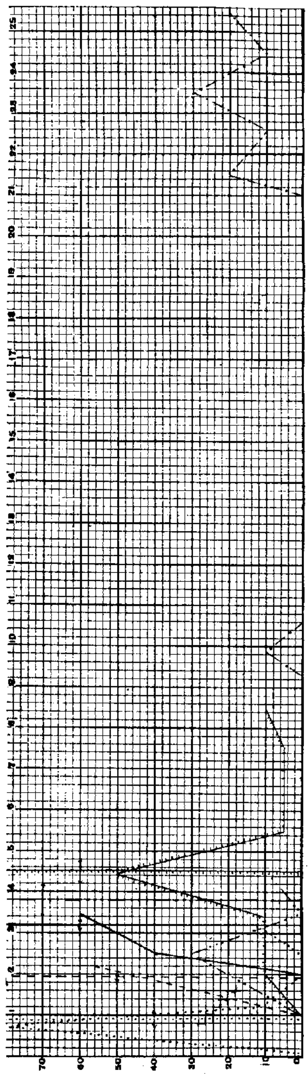

Fig. 9 Showing the results of tests to discover whether the resistance to N/400–500 sodium cyanide measures the metabolic rate of Asellus. Abscissae give time with each space worth twelve minutes; ordinates show the percentage dying each hour with each space worth 2 per cent. For details see p. 188.

the metabolic rate of Asellus. The results of this inquiry are shown in figure 9. In this figure the broken line gives the survival time of 7 small isopods, averaging 4.3 mm. long, in N/400 concentration. This is to be compared with the unbroken line which gives the resistance of 5 isopods that averaged 7.1 mm. long. The temperature in both cases was 24°C. The vertical lines give averages and the arrows show the extent of the probable error which here is less than a fourth of the difference.

The dotted line gives the resistance of 10 isopods 5.6 mm. long in N/500 solution whose temperature had been raised 6 to 8°. This is to be compared with the dot-dash line which gives the survival time of the same number of isopods 5.8 mm. long in the same solution strength whose temperature had been lowered 16°.

The dash-three-dot-dash line represents the survival time of 10 isopods which were stimulated by shaking. The average length of these animals was 5.8 mm.; the temperature 24°; the solution strength N/500. Shaking greatly hastens the loss of equilibrium but after the isopods quit moving, the effect is almost nil. This curve is to be compared with the cross barred line which shows the resistance of 20 isopods under conditions identical with the last but which were not stimulated. The probable error of the averages in this connection is about the same as the difference and taken alone would be meaningless but it supports the other curves at least to the extent that the difference is in the same direction.

We have here good evidence that young isopods and those at a higher temperature have a shorter survival time in cyanide than older animals or those at a lower temperature, and indications that stimulation by shaking also increases the susceptibility. If the cyanide is a measure of the metabolic rate of Asellus these results are logical.

Effect of potassium chloride on resistance to sodium cyanide

Since potassium chloride was the most efficient reagent found for increasing the positiveness of the rheotactic response the effect of this salt on susceptibility to sodium cyanide would

give the best evidence obtainable as to whether or not a change in the metabolic state accompanied the increase in positiveness. The extent of rheotactic change is shown in part I of table 5. Of the 49 isopods tested only one had its positiveness decreased by the 7–28 minute treatment with N/10 solution. The average rheotactic response was increased from 10 to 74 per cent positive. Comparison of the survival time with that shown in part II gives the effect of the treatment with potassium chloride upon the susceptibility to N/400 sodium cyanide. The resistance of 19 highly negative, untreated isopods was 6.1 (±0.3) hours and of the 49 untreated isopods 4.12 (±0.2) hours. The difference is approximately two hours which is four times the probable error and therefore statistically significant.

These results are also graphically shown in figure 10 in which the solid line gives the resistance of the 49 isopods after being made positive with potassium chloride and the broken line gives that of the 19 untreated animals. The height above the base line gives the number of isopods dying each hour. The vertical lines again show averages and the double pointed arrows give the extent of the probable error.

Effect of calcium chloride upon resistance to cyanide and upon carbon dioxide production

Opposed to potassium chloride, calcium chloride was the most effective depressing salt found. Its effect on the metabolic rate may be judged both by its effect on resistance to cyanide and on carbon dioxide production.

Seventy-one highly positive isopods were treated with calcium chloride long enough to cause a reversal in rheotaxis and then gave a resistance to N/400 sodium cyanide of 6 hours 34 minutes (± 4 min.) while 27 highly positive, untreated isopods gave an average resistance to the same strength of cyanide of 5 hours 32 minutes (±13 min.) The difference here is 3.7 times the probable error. It was not thought necessary to carry this series further because of work done with Dr. Tashiro (Allee

TABLE 5

Showing the effect of N/10 *KCl upon rheotaxis and survival time in* N/400 *NaCN*

I. Isopods treated with KCl until highly positive to a water current. Temperature 23 to 60°C.

RHEOTAXIS AT START			AFTER TREATMENT				TIME IN KCl IN MINUTES	SIZE IN MM.	SURVIVAL TIME IN NaCN.
+	−	α	+	−	α	0			
5	95		65	35			15.0	7.0	1:20
5	95		65	35			15.0	7.0	2:15
5	95		65	35			15.0	7.0	3:15
5	95		65	35			15.0	7.0	3:15
40	60		60	40			15.0	7.0	1:15
12	82	6	100				15.0	8.0	2:14
12	82	6	100				15.0	6.0	2:14
12	82	6	60	20	20		15.0	6.0	2:14
	100		100				7.0	5.0	6:09
	100		80	20			7.0	5.0	2:44
	80	20	20		80		7.0	6.5	3:09
	40	60	50	50			14.0	5.0	4:10
40	60		100				14.0	5.0	5:10
	100		100				14.0	5.0	7:40
	100		80	20			12.0	5.0	3:15
	100		100				12.0	6.0	1:50
80	20		100				11.0	5.0	1:25
20	80		60	20	20		11.0	6.5	2:55
	100		80			20	11.0	6.5	1:55
	100		80		20		11.0	7.0	2:10
	100		33		33	34	9.0	5.0	2:07
	100		100				13.0	7.0	2:54
	20	80	33		67		9.0	6.0	3:07
	100		100				14.0	7.0	2:27
40	40	20	20	80			13.0	5.5	6:12
	40	60	40	60			13.0	5.5	3:42
	80	20	80	20			13.0	6.0	3:12
	60	40	100				13.0	6.0	6:12
	20	80	20	20	40	20	13.0	6.5	1:32
20	80		100				8.0	5.0	3:50
	100		70	10	20		8.0	6.0	12:00
	100		60		40		28.0	6.0	2:55
20	80		50		25	25	18.0	5.0	2:54
40	60		100				14.0	6.0	7:34
80	20		100				14.0	6.0	10:30
20	80		60	40			14.0	7.0	5:34
	100		60	20	20		12.0	7.0	4:52
	100		80	20			12.0	8.0	4:30
	100		100				22.0	10.0	3:52

TABLE 5—*Continued*

RHEOTAXIS AT START			AFTER TREATMENT				TIME IN KCl IN MINUTES	SIZE IN MM.	SURVIVAL TIME IN NaCN
+	−	α	+	−	α	0			
	100		100				12.0	6.0	3:52
	100		60	40			12.0	7.0	3:52
	100		100				12.0	5.5	6:47
	100		80	10	10		17.0	6.5	3:30
40	40	20	70	10	20		12.0	7.5	3:17
	100		60	20	20		12.0	7.5	2:47
	100		100				22.0	5.5	3:47
	100		60	40			15.0		5:02
	100		70	20	10		25.0		13:00
	100		100				28.0	7.0	5:30
Ave. 10	82	8	74	15	9	2	13.8	6.4	4:07 ±12

II. Negative, untreated isopods. Temperature 17 to 23°C.

RHEOTAXIC TEST			TIME IN KCl IN MINUTES	SIZE IN MM.	SURVIVAL TIME IN NaCN
+	−	α			
	100		0	7.5	4:30
	100		0	6.0	5:30
	100		0	5.0	5:30
	100		0	5.5	6:35
	100		0	5.0	7:05
	100		0	6.5	7:05
	100		0	5.0	7:30
	100		0	10.0	7:55
	100		0	7.0	10:05
	100		0	8.0	10:55
20	80		0	6.0	7:50
20	20	60	0	7.0	4:18
	100		0	6.0	8:32
	100		0	6.5	5:45
	100		0	7.0	7:20
	100		0	7.5	3:02
	100		0	6.5	5:43
	100		0	7.0	4:18
	100		0	9.0	5:18
Ave. 2	95	3	0	6.7	6:06 ±18

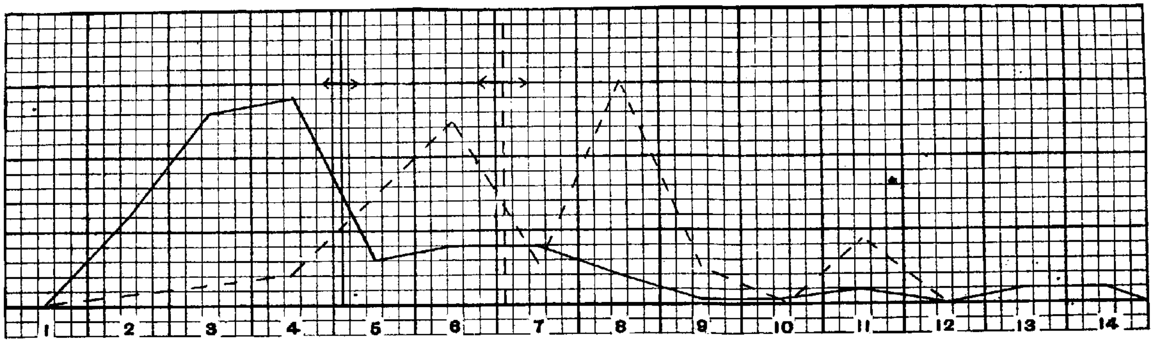

Fig. 10 Showing the resistance to N/400 sodium cyanide of isopods which had had their rheotactic reaction reversed (unbroken line) by treatment with potassium chloride and of untreated isopods (broken line). Abscissae give time and the ordinates show the percentage dying each hour. For details see p. 189.

and Tashiro '14) upon the effect of calcium chloride upon carbon dioxide production in Asellus. A part of these results are republished here:

In brief the experiments were as follows: Two isopods of approximately the same size were tested for their relative rate of carbon dioxide production in the Biometer. The isopod having the lower rate of carbon dioxide output was taken as a control and was again tested for the rheotactic reaction and then left in conditions to which it was acclimated while the other was treated. The second individual which had the higher rate of carbon dioxide production was placed in a 0.2 mol. solution of calcium chloride until the tendency to give a positive rheotactic reaction was markedly reduced. Then the rate of carbon dioxide production of the two was again tested in the Biometer.

In both pairs tested the isopod with the higher rate of carbon dioxide production at the first test in the Biometer had also given the higher percentage of rheotactic responses, but after being treated with calcium chloride for 25 to 36 minutes it came to be less positive in its rheotactic reaction, and also gave less carbon dioxide and was less susceptible to potassium cyanide than the control individual. In other words the calcium chloride (0.2 mol.) decidedly decreased the rate of metabolism of the isopods and also reduced their tendency to give a positive rheotactic reaction.

Effect of cane sugar on resistance to potassium cyanide

Fifty-four isopods that gave an original average rheotactic response 33, 54, 9, 1 per cent positive, negative, indefinite, and zero were treated with M/2 cane sugar until they gave an average reaction of 18, 24, 52, 6. Forty-eight of these were immediately killed in N/1000 potassium cyanide and gave an average survival time of 8 hours 23 minutes ($\pm$31 min.). Eighty-six isopods that gave an average rheotactic response of 34, 57, 9 per cent respectively resisted the same strength of cyanide for an average of 5 hours ($\pm$7 min.). This is a difference of 3 hours 23 minutes which is over five times the probable error and undoubtedly significant. Since the cane sugar probably acted by removing water (Peters '04 for Stentor) we have here excellent evidence that such conditions depress the rate of oxidations in Asellus which corresponds to the results reported by Riddle ('14) for the pigeon's egg.

TABLE 6

Showing the effect of calcium chloride upon carbon dioxide production and rheotaxis in isopods. The survival time in potassium cyanide is added for comparative purposes. The isopods were first tested for rheotaxis, then two of approximately the same size were taken for determination of their carbon dioxide output in the biometer. The one of these that gave the least carbon dioxide was taken as a control, its rheotactic reaction was again tested and it was allowed to stand in water to which it was accustomed while the other was treated.

*The second isopod, the one giving the most carbon dioxide, was placed in a **0.2** mol. solution of calcium chloride until the positive rheotactic tendency was markedly decreased. Immediately afterward the carbon dioxide production of the two was again compared in the biometer.*

ISOPOD NO. 30	ISOPOD NO. 169
Rheotaxis test, 11:55 A.M. Temp. 20 50% +, 50% −; Efficiency, 2.1 Tested in Biometer 1:47–2:00 P.M. Temp. 23.5 Less CO_2 than No. 169 Rheotaxis test 2:00 P.M. 70%+, 20%−, 10%, Efficiency, 2.25	Rheotaxis test, 12:25 P.M. Temp. 20 90%+, 10%−; Efficiency, 2.1 Tested in Biometer 1:47–2:00 P.M. Temp. 23.5 More CO_2 than No. 30 Put in 0.2 Mol. $CaCl_2$ 2:05 P.M. Rheotaxis test 2:07 P.M. 80%+, 20%−; Efficiency, 1.6 Rheotaxis test 2:27 P.M. 40%+, 20%, 40%; Efficiency, .95 Taken from $CaCl_2$ 3:43 P.M. In $CaCl_2$ 36 minutes
Tested in Biometer 3:44–3:57 P.M. More CO_2 than No. 169 Survival time in 0.001 Mol. KCN 2 hours, 20 minutes ♂, 4.5 mm. long	Tested in Biometer 3:44–3:57 P.M. Less CO_2 than No. 30 Survival time in 0.001 Mol. KCN 3 hours, 10 minutes ♂, 5.0 mm. long

ISOPOD NO. 171	ISOPOD NO. 84
Rheotaxis test 12:25 P.M. Temp. 20 10%+, 90%−; Efficiency, 2.4 Tested in Biometer 2:49–3:05 P.M. Little CO_2 given off Less CO_2 than No. 84 Rheotaxis tested 3:50 P.M. 60%+, 40%−; Efficiency, 2.0	Rheotaxis test 12:00 M. Temp. 20 30%+, 60%−, 10%; Efficiency, 2.6 Tested in Biometer 2:49–3:05 P.M. Little CO_2 given off More CO_2 than No. 171 Put in 0.2 Mol. $CaCl_2$ 4:02 P.M. Rheotaxis tested 4:07 P.M. 10%+, 40%, 50%; Efficiency, 0.9 Taken from $CaCl_2$ 4:27 P.M. In $CaCl_2$ 25 minutes
Tested in Biometer 4:35–5:12 P.M. More CO_2 than No. 84 Survival time in 0.001 Mol. KCN 1 hour, 35 minutes ♂, 5.5 mm. long	Tested in Biometer 4:35–5:12 P.M. Less CO_2 than No. 171 Survival time in 0.001 Mol. KCN 2 hours, 55 minutes ♂, 6.0 mm. long

Since the most efficient stimulating and depressing salts found affect the metabolic rate of the isopods as measured by their resistance to the cyanides and (calcium) by carbon dioxide production the results of these experiments support earlier work on this subject which demonstrated that Asellus with a high rate of positive rheotaxis have a relatively high rate of metabolism and those with a low degree of positiveness tend to have a low rate of metabolism. Lillie ('09) gives evidence to show that the primary action of pure sodium and potassium chloride solutions is to increase, and that of magnesium and calcium chlorides, to decrease permeability in Arenicola larvae and that these stimulate and depress, respectively, the muscular activity of these animals. He concludes that this increase in permeability is in itself sufficient to account for the liberation of energy which is the essential consequence of stimulation. Whether or not the relationship between these salts and rheotaxis can be fully explained on such a basis is a matter for further experimentation.

V. SUMMARY OF EXPERIMENTAL RESULTS

The chlorine salts of the alkali metals affect the rheotactic reaction of Asellus communis in such a way as to suggest that there is a relation between the chemical activity of these cations and their effect on rheotaxis of isopods. Potassium is the most effective in increasing the positiveness of the reaction, with rubidium a close second, p. 167.

The relative toxicity of these cations does not run parallel with their stimulating power but resembles the relative favorableness in preserving the activity of frog nerves and muscles p. 170.

The anions of the most stimulating cation, potassium, affect the rheotactic reaction but their effectiveness does not run parallel with their chemical activity and the relative toxicity, while similar, is not exactly the same as the stimulating power, p. 172.

Any chemical in the concentrations used will cause a decrease in the positive rheotactic reaction, but the chlorine salts of

calcium and strontium cause this decrease usually without a preliminary stimulation. Magnesium chloride while in the main similar in action often causes preliminary stimulation and barium chloride is still more stimulating, resembling the alkali metals in its effect, p. 173.

In the cations such as potassium which are highly stimulating the depression is a toxic effect while in depressing cations as calcium, rheotaxis is depressed long before toxicity symptoms appear, p. 174.

There is a marked antagonism between the effect of potassium and calcium chlorides and a less marked one between the chlorides of sodium and magnesium, p. 175.

Both acids (H ions) and alkalies (OH ions) in the concentrations used generally decrease the percentage of positive responses given, p. 177.

Once distilled water gave some evidence of causing Asellus to become more positive in their rheotactic reaction, though pure water was quite toxic, p. 181.

Cane sugar decreased the positive rheotactic reaction probably by extracting water and the action of the once distilled water may be due to water intake, p. 183.

The results obtained with salts are not osmotic effects because equimolecular solutions of different salts with approximately the same osmotic pressure may have opposite effects and because cane sugar in M/2 solution was a less effective depressant than N/10 calcium chloride although its osmotic pressure is over three times as great, p. 185.

Susceptibility to sodium cyanide N/400 or N/500 measures the rate of metabolism of Asellus probably by limiting the oxygen consumption, p. 188.

Measured in this way, potassium chloride when increasing positive rheotaxis also increases the rate of isopod metabolism, and calcium chloride and cane sugar decrease both positive rheotaxis and the metabolic rate. Calcium chloride also decreases the carbon dioxide output, p. 189.

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THE EFFECT OF RADIUM RADIATIONS ON THE RATE OF CELL DIVISION

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The radiations of radium produce two distinct types of effects on living matter, depending on the intensity of the radiation. When the exposure is intense or prolonged many abnormalities result. In the unfertilized or freshly fertilized egg they produce a marked cytolysis, in which the water holding power of protoplasm is markedly increased; with this condition is usually associated a derangement in the mechanism of mitosis. As a result, the formation of polar bodies is interfered with or suppressed, wholly or partially, or multipolar figures are produced. The embryo is always abnormal in the case of Nereis and Arbacia. Oscar Hertwig found that if the unfertilized frog egg is radiated up to a certain point the resulting embryo after insemination with normal sperm is abnormal, but if the radiation is very intense development is haploid, the egg nucleus having been rendered incapable of playing its usual part in fertilization.

This phase of the subject has lead to no significant results thus far, perhaps on account of the difficulty of interpreting the very diverse effects. The disturbance of the chromatin in division has lead Gunther Hertwig to draw interesting conclusions as to the functions of chromatin in heredity, but this is aside from the general problem of the effect of radium. In his experiments he employed radium as an effective means of injuring the sperm, but his conclusions would probably have been the same had he used any other means of disturbing the chromotin without interfering seriously with the protoplasmic activities of the sperm.

When the exposure to radium is brief, or the amount used is small, an entirely different kind of phenomenon appears. This consists in a change in the division rate of cells, which is not followed by any apparent abnormality. Many observers have noted this effect but have made little attempt to analyze the problem. In most instances they have described a retardation in the rate of division but occasionally they have noted an acceleration.

Richards ('14) studied the effect of X rays on the cleavage of the eggs of the snail Planorbis. As X rays are similar to the gamma rays of radium his results have a direct bearing on the problem discussed in this paper. He found that brief exposures produced an acceleration in the rate of cleavage, but this effect was only temporary since after the first cleavage the treated eggs were retarded. The greatest effect was produced when exposures were made during the metaphase. During the resting stage there was very little response one way or the other.

The experiments described in this paper were carried on to find out more exactly the conditions under which acceleration or retardation may be produced by the action of radium radiations on the fertilized eggs of Arbacia.

METHODS

In conducting experiments on the rate of cell division it is of prime importance to keep the temperature of the sea water constant throughout. To this end I carried out each experiment in the following manner. Syracuse watch glasses were kept in running sea water for thirty minutes to bring them to an even temperature. They were then quickly dried without being touched by the hands, and filled with a measured amount of sea water. The Arbacia eggs, which had been freshly fertilized were now added in measured quantities. It was found necessary to use only a few eggs at a time for a very little overcrowding itself changes the rate of division and produces abnormalities. One watchglass was placed under the radium tube

which contained 50 mg. of pure bromide, equivalent to 23.4 mg. of element. The control eggs were placed in a watchglass beside the others but separated from them by a thick sheet of lead. Frequent tests showed that there was no appreciable difference in the temperature of the two lots of eggs during the entire experiment. This point indicated that the radiations from this amount of radium are not sufficient to produce any marked temperature change.

The radium tube was held in a device so arranged that the tube could be held at any desired distance above the eggs. As there was no screen of any kind interposed between the radium and the eggs it is assumed that all of the rays emanating from the tube, namely the beta and gamma rays, were able to reach the eggs. After exposure the eggs, with the controls, were observed almost continuously until after the first cleavage had been completed.

The amount of acceleration or retard is estimated by comparing the time elapsing between fertilization and cleavage in the controls and the radiated eggs. Thus in some experiments the controls began to divide 60 minutes after insemination, and the radiated eggs, in 50 minutes. The difference of 10 minutes is about 16 per cent of the 60 minutes elapsing before cleavage occurred in the controls.

Exposures were made during five periods of the cell's activity, namely, between 5 and 25 minutes after insemination when the germ nuclei are approaching each other; second, during the prophases which occur from about 25 to 35 minutes after insemination; third, during the metaphase which appears in about 35 to 50 minutes; fourth, during the telophases (50 to 65 minutes after insemination); and finally during the resting stages following the first cleavage. Naturally the temperature of the air hastened or retarded this rate, so that in some instances the exposures had to be shorter than the limits mentioned. In each experiment the distance between the radium and the eggs was varied from $\frac{3}{8}$ inch to $2\frac{1}{4}$ inches.

OBSERVATIONS

The experiments related below are typical of a large number, and only those are recorded which have been verified by repeated trials.

1. Exposure during the period in which the germ nuclei are approaching and fusing with each other. Five to twenty-five minutes after insemination.

Radium placed $\frac{3}{8}$ inch above eggs

Length of exposure	Result
5 minutes	Slight acceleration
10 minutes	5 per cent acceleration
15 minutes	Slight acceleration
20 minutes	No acceleration

Radium placed $\frac{3}{4}$ inch above eggs

10 minutes	Slight acceleration
15 minutes	Slight acceleration
20 minutes	No acceleration

If the radium is placed $1\frac{1}{2}$ or $2\frac{1}{4}$ inches above the eggs there is little evidence of any change. These results were constant and clear cut, even when the amount of change in division rate was small.

Under these conditions it is clear that unless the exposure is intense there is little or no response. These results were anticipated for I have found in previous experiments that freshly fertilized or unfertilized eggs are not very susceptible even to intense exposures. However, if the radiation is prolonged the whole organization of the egg undergoes a profound change, and subsequent development is very abnormal. Inasmuch as I was not trying to produce abnormalities, I did not use very intense radiations.

2. Exposure made during the prophases of mitosis. Twenty to thirty-five minutes after insemination.

Radium placed $\frac{3}{8}$ inch above eggs

Length of exposure	Result
5 minutes	Slight acceleration
10 minutes	10 per cent retard
15 minutes	15 per cent retard

Radium placed ¾ inch above eggs

10 minutes	5 per cent retard
15 minutes	10 per cent retard

Radium placed 1½ inches above eggs

10 minutes	5 per cent acceleration
15 minutes	Slight acceleration

At a distance of 2¼ inches the radiations produced no decisive effects.

From these experiments it appears that during the prophases the egg is more susceptible than during the preceding period. An exposure which, during the first period produces an acceleration, here produces a retard, indicating that the optimum amount of radiation has been exceeded.

3. Exposure made during the metaphase . Thirty-five to fifty minutes after insemination.

Radium placed ⅜ inch above eggs

Length of exposure	*Result*
5 minutes	5 per cent retard
10 minutes	15 per cent retard

(Longer exposures produced some abnormalities)

Radium placed ¾ inch above eggs

5 minutes	Slight retard
10 minutes	10 per cent retard

Radium placed 1½ inches above eggs

5 minutes	No change
10 minutes	5 per cent acceleration
15 minutes	10 per cent acceleration

At 2½ inches exposures up to 15 minutes produced no clear cut effects.

It is evident that the results noted here are of the same order as those seen in the preceding experiment, but they are of greater magnitude.. It may be said therefore that eggs in this condition are more susceptible than before.

4. Exposures made during the telophases. Fifty to sixty-five minutes after insemination.

Radium placed $\frac{3}{8}$ inch above eggs

Length of exposure	Result
5 minutes	No change
10 minutes	5 per cent retard
15 minutes	5 per cent retard

Radium placed $\frac{3}{4}$ inch above eggs

10 minutes	Slight acceleration
15 minutes	5 per cent acceleration

At the greater distances there was no clear evidence of any change. These results indicate that eggs during the telophase are not as responsive as they are during the metaphase, but are more like the eggs in the prophase of mitosis.

5. Exposures made during the resting stage of the nuclei were followed by no decisive changes unless the radiation was intense and prolonged, in which case the eggs developed abnormally, as would be expected. The responsiveness of the egg has now reached its minimum. This result agrees very well with the results of exposures made on tissues in which little cell division is taking place. Unless the cells are dividing rapidly radiation produces little result except when it is very intense.

In all of these experiments it is assumed that each egg receives an equal amount of radiation. Probably this is not strictly true, especially when the distance through which the radiations pass is small, for the distance from the tube to the eggs directly under it was somewhat less than the distance to the eggs further from the center of the watch glass. The fact that the source of radiation is not a single point but a tube 1 cm. long would also make a difference. However the inequality in the amount of radiation received by the eggs is very small and is constant for each experiment, so the results are not obscured.

The amount of radiation which falls on the eggs decreases inversely with the square of the distance through which it passes. Thus if we consider $\frac{3}{8}$ inch as a unit distance, the energy recevied at $\frac{3}{4}$ inch is one-fourth of that received at unit distance. At $1\frac{1}{2}$ inches it is one-sixteenth, and at $2\frac{1}{4}$ inches, one thirty-sixth of the unit amount. Theoretically we should expect the re-

sults to vary in these ratios, but under the conditions of the experiment it is difficult to estimate in these terms. It was found impossible to expose eggs at $2\frac{1}{4}$ inches for thirty-six times as long as at $\frac{3}{8}$ inch for during that prolonged period the eggs pass through all of their phases of division at least twice.

A further difficulty is found in the fact that the beta rays, which are very active in producing changes in protoplasm, as I have shown (Packard '15), are easily stopped by air so that at a distance of two inches or more very few of them reach the eggs.

A few experiments were made in which the eggs were cooled in a freezing mixture so as to retard their rate of development, and the radium was applied for a longer time. But the results were not satisfactory as it was found difficult to hold the control eggs and the radiated eggs at exactly the same stage of development. It was also impossible to keep both lots at exactly the same temperatures throughout the experiment. Although some of the trials tended to confirm the expectations, the general results were not conclusive or reliable.

DISCUSSION

The radiations of radium produce effects in matter only when they are absorbed wholly or in part by it. The beta rays, because of their relatively low velocity are quickly stopped by many substances, the particles colliding with the molecules of the substance, and in many instances producing a marked ionisation of the molecules. As these rays are not homogeneous but are made up of particles projected with varying velocities, it follows that the slower particles are stopped more quickly than the more rapid ones and produce greater chemical effects. The fact that they affect protoplasm more vigorously than the rapid rays was shown in a recent paper (Packard '15). Whether all of the effects noted in those experiments were due to an ionisation of protoplasm is a point yet to be determined.

The gamma rays are exceedingly penetrating and are stopped completely only by thick sheets of the heavy metals. Some of them however are stopped by protoplasm, especially if the

layer of living tissue be of considerable thickness. When this stopping occurs, due to the violent encounters of the rays with the protoplasmic molecules, the gamma rays disappear and their energy is converted into that of beta particles which travel on in the direction of the original gamma ray. These secondary beta rays produce effects similar to those produced by the primary beta rays. The great penetrating power of the gamma radiations accounts for the fact that few of them are thus changed into beta rays in protoplasm and hence produce few changes in living matter unless an enormous number is allowed to act on it. X rays which are similar to these radiations produce their characteristic effects by means of the secondary beta rays which they generate. The effects produced by these two types of radiations are therefore comparable.

The amount of radiation which is stopped by matter depends on the density of the elements composing it. Roughly, the absorptive power varies with the square root of the atomic weight of the elements, and therefore follows closely the grouping of the elements in the periodic table. From this it is clear that the light elements composing protoplasm, such as carbon, oxygen, hydrogen, and nitrogen have relatively low powers of absorption.

The absorption of radiations by compounds is equal to the coefficients of absorption of the elements composing them. That is, the stopping power of a compound depends on the number and atomic weight of its constituent atoms. This rule applies to all of the compounds studied thus far. Borodowsky showed that the absorption of beta rays by liquids follows accurately an additive law, and does not depend on the concentration or chemical dissociation of the liquids. The amount of radiation which an organic liquid can absorb can be deduced from a knowledge of its constituent elements. From this it follows that the amount of absorption is not influenced by the molecular structure of substances. Applying these facts to the protoplasm of the Arbacia egg, it is evident that the protoplasm will always stop a definite amount of the radiation provided that the chemical make up of the eggs remains unchanged. It

is also evident that in these experiments no profound change took place which involved a dropping out of any of the elements present in the protoplasm at the beginning of the experiment, since the eggs developed perfectly normally after the treatment. This fact is important in the explanation of the results here presented, for at first sight they seem to indicate that the reverse is true, namely, that at certain periods of their activity the eggs are more absorptive to the rays than at others. Inasmuch as this cannot be true we must look to some physiological changes in the egg which render it more susceptible at one time than at another.

When protoplasm is intensely radiated enough energy is absorbed to bring about a marked physical and chemical change. This may consist in partial ionisation of the molecules, or in the breaking down of compounds present. Schwarz has shown that when an egg, rich in yolk, is intensely radiated, the lecithin is decomposed into cholin which acts as a poison. It is doubtful whether this explanation can suffice to account for some of the effects reported, since not all of the cells which are injured contain lecithin in large amounts. This is particularly true in the case of tumor cells which contain, according to recent analyses, no more lecithin than the surrounding normal cells which are uninjured by the radiation, although the tumor cells are obviously affected.

Other effects of severe radiation, such as cytolysis, have already been mentioned. When the unfertilized Nereis egg is radiated the cell wall is greatly weakened and the vitelline membrane altered to such an extent that it allows many sperms to enter. Prolonged exposures of the sperm cause in the chromatin a change which shows itself in the fragmentation of the sperm nucleus after it has entered the egg (Hertwig '12).

Gunther Hertwig has suggested that radium acts directly on the chromatin. That chromatin is more affected by equal amounts of radiation is undoubtedly true, but it is also true that protoplasm is actually affected. I have elsewhere (Packard '14) pointed out some objections to this theory. In terms of absorptive powers it assumes that chromatin is better able to

stop the radiations than protoplasm. This is probably true, since the former substance contains more phosphorus and iron than the latter. But the difference in composition is not so great as to account for the striking differences in response.

Furthermore, the changes in the apparent susceptibility of chromatin during the different phases of mitosis cannot be explained by the statement that its chemical constitution has altered so as to render it more or less absorptive. The time elapsing between the prophase and the metaphase is so brief that we cannot imagine any profound chemical change, involving a dropping out of elements, to occur. It is obvious therefore that to account for the observed phenomena we cannot assume that the chromatin is more able to stop the rays, or that it varies in its ability to stop them, at different periods of its activity. It is necessary to take into account the physiological processes occurring during mitosis.

During the periods in which the radium was applied to the eggs two phenomena which are of interest in this connection occur. In the first place the chromatin, which, during the resting stage, or the stage previous to the fusion of the germ nuclei, is diffuse and semi liquid, becomes condensed and gelatinous in the metaphase. After this time it gradually becomes more and more diffuse up to the period of the resting stage. On account of this increase in its density we might suppose that it becomes more absorptive to the rays. But a consideration of other experiments shows that this cannot be true. The sperm, in which chromatin is condensed to the greatest degree, is far more resistant to radiations than is the egg in which the chromatin is diffuse. I have exposed Nereis sperm for five hours yet at the end of that time they were not only actively motile but were able to bring about normal fertilization of the fresh egg. The fact that in the egg and the sperm there is an equal amount of chromatin, yet the egg is much more easily affected than the sperm, argues that something beside chromatin is involved.

The second phenomenon which occurs during this period is the breaking down of the nuclear wall with the liberation into the egg protoplasm of the nuclear sap. This is followed by a

marked increase in the rate of oxidation taking place in the egg. This increase is due to the increased activity of the oxidative enzymes, such as nuclease. The continuance of the process of mitosis depends on these enzymes, for if oxygen is withdrawn cell division stops. Mathews has suggested that

> Autolytic enzymes also evidently become active, either because they are set free from the nucleus, or because the nuclear materials activate, directly or indirectly, the inactive enzymes of the cytoplasm. . . . . Since during cell division these enzymes are set free and at the same time the chromatic elements are plainly losing substance, it is possible that these two facts should be correlated and that the conclusion drawn that in the resting condition of the nucleus enzymes of various kinds stick to, or combine with, the nucleic acid and are thus accumulated, made resistant, more stable and inert, and that during caryokinesis and possibly at other time also, they are split off from the acid, become free in the sap, enter the cytoplasm and rejuvenate the cell by digesting its accumulated colloidal material.

If it is granted that the phenomena of mitosis are dependent on the activity of intracellular enzymes, then it is clear that if these enzymes can be stimulated or retarded in their activity the result will be an acceleration or a retardation in the rate of cell division. I believe that the radiations of radium are able to change the rate of enzyme action, and that this is at least a partial explanation of the results recorded in these experiments.

I have already reviewed briefly the literature on the effect of radium on enzyme action. There is some lack of agreement in the results obtained by various observers. The radiations are stated to retard the digestive action of pepsin and trypsin, to accelerate it, and to have no effect whatever. Similar statements have been made regarding other enzymes. Richards ('14) has shown how these contrary results may be explained. Using X rays on pepsin and diatase he found that a short radiation has the effect of accelerating their activity, while a longer radiation inhibits it. "Between these two strengths lies a point at which radiation is non effective." These results on extracted enzymes are exactly similar to the results given in this paper. A short radiation brought about a stimulation, while a longer one produced a retard. Between these two limits

there was a strength of radiation which produced no noticeable affect, i.e., the initial acceleration was overcome by a subsequent retard. These similarities strengthen the view that in living eggs the changes in division rate are brought about by changes in the rate of enzyme action.

Assuming this hypothesis to be the correct explanation, it remains to explain why an acceleration or retard of the enzyme action can account for the differences in response under similar exposures during different phases of mitosis. Mathews suggests that while the nuclear wall is intact the enzymes are restricted in their action, but that they are able to produce oxidations as soon as the nuclear wall breaks down, that is, just before the metaphase. Then a mild radiation, during the prophase, although it activates the enzymes, results in little change in the egg since they are unable to act to advantage. But when the nuclear wall disappears and, under normal condition, active oxidation takes place, a slight radiation activates them as before, but because they are able to react with the protoplasm more vigorously than before, their activation leads to a more marked acceleration. If the radiation is more intense a retard results, that is, the optimum radiation has been exceeded and the enzymes are injured. These results are analogous to those obtained by treating protoplasm with poisons. A small amount of CO_2 accelerates muscular action while a larger amount retards it.

This hypothesis throws light on the well recognized fact that actively dividing cells are more susceptible than those which are not undergoing mitosis. Slow growing tumors are not susceptible (unless they are superficial and can be so intensely radiated that the protoplasm is injured) while rapidly proliferating tumor cells are very susceptible.

SUMMARY

Arbacia eggs exposed to a brief but intense radiation during the period when the germ nuclei are approaching each other are accelerated in their rate of cell division. Less intense radiation produces less acceleration.

Exposures made during the prophase result in an acceleration unless they are prolonged, when a retardation ensues.

During the metaphase the same phenomena appear but to a greater degree.

During the telophase the effects are much the same as in the prophase.

Eggs exposed during the resting stage are not easily affected.

The power of the protoplasm and chromatin to absorb the radiations does not change during these periods.

The differences in the density of the chromatin during the different phases of mitosis do not affect its absorptive power.

During the metaphase when the eggs are most responsive to radiations oxidations take place through the activity of enzymes. If these enzymes are accelerated or retarded the effect is to accelerate or retard the rate of cell division.

Experiments indicate that radiations produce these effects on extracted enzymes.

It may be inferred therefore that the endoenzymes are affected in the same way and that changes in the rate of cell division, following radiation, are due to the direct action of the radiations on them.

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CHROMOSOME STUDIES ON THE DIPTERA II. THE PAIRED ASSOCIATION OF CHROMOSOMES IN THE DIPTERA, AND ITS SIGNIFICANCE

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EIGHT PLATES

CONTENTS

INTRODUCTION

Attention was first called to the pairing of chromosomes in the Diptera by Miss N. M. Stevens during 1907 and 1908 in connection with studies upon the heterochromosomes of insects (Stevens '07, '08). Although primarily concerned with the heterochromosomes and maturation phenomena, Stevens nevertheless found the paired association of chromosomes, in the nine species she studied, so conspicuous as to warrant the statement that, "perhaps the most interesting point in the whole study is the pairing of chromosomes in cells somewhat removed from the sphere of the reduction process. This was found to occur in the ovarian follicle cells, the spermatogonia and some embryonic cells. This is not an occasional phenomenon, but one which belongs to every oogonial and spermatogonial mitosis"

(Stevens '08, p. 372). In a later paper on "The chromosomes in the germ-cells of Culex" (Stevens '10, p. 215), corresponding phenomena called forth a similar statement to the effect that "perhaps the most interesting point in the history of the germ-cells of Culex is the fact that, as in the Muscidae, pairing or synapsis, occurs in connection with each spermatogonial and oogonial mitosis as well as in anticipation for maturation." Although only able to study somatic mitoses to a very limited extent, Stevens surmised that, "it may therefore be true that pairing of homologous chromosomes occurs in connection with each mitosis throughout the life history of these insects" (p. 215). Now this would be a very important point to establish, as Stevens realized, and she doubtless would have followed it up had it not been for her untimely death in 1912. Most unfortunately, however, her work on the Diptera was stopped at its very beginning and many promising questions suggested by it have remained uninvestigated.

Nothing further appeared on chromosomes of the Diptera until 1914 when three papers were published, one by the author on Drosophila chromosomes; the others on the chromosomes on Culex pipiens, one by Miss Taylor, and one by Lomen. Both of the latter took exception to Stevens' conclusions that the chromosomes are paired in Culex and other Diptera, on the ground that the chromosome pairs which she described were really only precociously split univalent chromosomes. Their evidence on this point, however, is very inadequate, and their conclusions are surely erroneous (see pp. 244 and 245).

The purpose of the present paper is to describe in some detail the phenomena involved in 'chromosome pairing' in the Diptera, and to consider their bearing on current theories respecting the nature of the chromosomes and their rôle in heredity. Because of their remarkably definite paired association the chromosomes of the Diptera are especially suitable for studies on the relationships between individual chromosomes and on the qualitative characteristics of chromosomes as indicated by their behavior, but as I have mentioned in a previous paper (Metz '14) the technical difficulties involved in an extensive

cytological study of these insects have caused them to be generally avoided by cytologists. These difficulties, however, may very largely be overcome by care and persistence. Although certain principles must be observed in making preparations, the task is mainly one of securing and preparing enough specimens to get material in the proper stages and in sufficient quantity for study. No more difficulty is experienced in studying the nuclear phenomena, when the proper material is secured, than is the case in other insects; indeed the chromatic elements in the flies, when well prepared, appear with a brilliancy that is surpassed by very few objects.[1]

The observations included here are concerned chiefly with chromosomal behavior in somatic cells and in germ-cells outside the sphere of maturation. These cells I shall briefly term 'diploid' cells, in distinction to oocytes and spermatocytes. Since all of the 'diploid' cells agree in respect to the phenomena dealt with, no confusion should arise from such a terminology. Phenomena associated with the maturation processes are considered only in so far as they bear directly upon those in 'diploid' cells. Likewise the relationships between the chromosomes in different species of flies are only briefly considered. I hope to return to both of these questions in subsequent papers.

In order to facilitate the treatment of the subject matter I will outline at once the main points considered in the paper, and will indicate in advance some of the conclusions attained. This may best be accomplished by taking account of certain genetic hypotheses which intimately involve the chromosomes and which have furnished the occasion for this investigation.

These hypotheses are all contained in one comprehensive theory which has recently been brought into prominence by the rapid development of Mendelism. According to this theory the chromosomes are complex, accurately differentiated bodies whose organization and behavior are directly correlated with the genetic factors located in them. In any biparental organism, the diploid chromosome group is composed of two equivalent,

[1] Except in maturation stages, which are often very unfavorable for study.

parental series (haploid groups), the individual members of which are respectively homologous and very similar to one another; and this involves the view that the chromosomes are present in bi-parental pairs (Montgomery, Sutton, Boveri). In addition it is supposed, in accordance with the conception of W. Roux that every chromosome contains a definite complement of serially arranged genetic factors, each responsible for one or more inherited characters—the complement of factors being the same or similar in homologous chromosomes (members of a pair) but different in non-homologous chromosomes. In order to explain the perpetuation of this duplex germinal constitution a process (reduction division) is assumed to occur during maturation whereby the members of each pair are separated from one another and segregated in different germ-cells.

From the cytological point of view the principal questions involved in this theory are as follows: 1) Can definite pairs of chromosomes really be distinguished? 2) If so, are the two members of a pair derived respectively from the male and female parents? 3) Are the two members of a pair actually similar to one another and qualitatively different from the others in respect to their physico-chemical constitution? 4) Do the two members of a pair actually separate from one another and go into different germ-cells during maturation?

Three of these questions, together with one other of a more strictly cytological nature—the question of synapsis—form the central points about which most of the facts considered in the present study may be grouped. The nature of the material prevents the detailed consideration of each question in the order given, but so far as possible the evidence is presented in accordance with this scheme. The evidence bears especially upon the first question, to which a definite affirmative answer is given. With respect to the second question judgment should, perhaps, be suspended until the genetic continuity of the chromosomes is established, but if this continuity be assumed, this question is likewise answered in the affirmative. Regarding the third question only indirect evidence is furnished, but this evidence lends support to an affirmative answer here also. The fourth

question is not directly involved in the present paper. In regard to the problem of synapsis the pairing phenomena in diploid cells, including final spermatogonia, clearly demonstrate that a side by side approximation of corresponding chromosomes (the essential feature of synapsis), actually does occur, although in this case it is not connected with maturation.

Throughout the course of this study I have profited greatly by the counsel of Prof. E. B. Wilson, under whose direction the work was begun, and to whom I have become increasingly indebted for many kindnesses.

MATERIAL AND METHODS

My observations are based upon a study of the chromosomes in about eighty species of Diptera, representing thirty-five genera and fifteen families, as given in the following synopsis.

ORTHORRAPHA

- Nemocera
  - Culicidae
    - *Culex pipiens* Linne.
- Brachycera
  - Stratiomyidae
    - *Ptecticus trivittatus* Say.
  - Asilidae
    - *Asilus sericeus* Say.
    - *Asilus lecythus* Walk.
    - *Asilus notatus* Wied.
    - *Asilus novae scotiae* Macq.
    - *Asilus sadytes* Walk.
    - *Ommatius marginellus* Fabr.
    - *Leptogaster badius* Loew.
    - *Erax aestuans* Linne.
    - *Erax rufibarbis* Macq.
    - *Dasyllis grossa* Fabr.
    - *Dasyllis thoracica* Fabr.
    - *Deromyia winthemi* Wied.
- Bombyliidae
    - *Anthrax lateralis* Say
    - *Anthrax sinuosa* Wied.
    - *Spogostylum simson* Fabr.

CYCLORRHAPHA

Syrphidae

Eristalis tenax Linne.
Eristalis bastardi Macq.
Eristalis aeneus Fabr.
Eristalis meigeni Wied.
Volucella obesa Fabr.
Mesogramma marginata Say.
Toxmerus annulatus Loew.

Acalypterae

Micropezidae

Calobata lasciva Fabr.
Calobata nebulosa Loew.

Sepsidae

Piophila casei Linne

Ortalidae

Chaetopsis fulvifrons Macq.
Camptoneura picta Fabr.
Euxesta stigmatius Loew.
Euxesta anonae Fabr.

Trypetidae

Euaresta melanogaster Loew.

Sapromyzidae

Physegenua vittata Macq.

Drosophilidae

Drosophila.—27 species, many undescribed, see text.
Cladochaeta nebulosa Coq.
Scaptomyza adusta Loew.
Scaptomyza graminum Fall.

Sciomyzidae

Neuroctena analis Fullen.

Calypterae

Anthomyidae

Homalomya spp.
Fucellia marina Macq.
Ophyra leucostoma Wied.

Muscidae

Calliphora viridescens Desv.
Calliphora erythrocephala Meig.
Musca domestica Linne.
Muscina stabulans Fall.
Phormia regina Meig.
Lucilia sericata Meig.
Pseudopyrellia cornicina Fabr.

Sarcophagidae

Sarcophaga falculata Pand.
Sarcophaga tuberosa serraceniae Riley.
Sarcophaga dalmatina Schin.
Sarcophaga bullata Park.
Ravinia communis Park.
Ravinia peniculata Park.

Preparations have been made from gonads of both sexes, and somatic tissues of various kinds. Almost all of the latter represent embryonic stages, including eggs, larvae and pupae. The former have been taken from larvae, pupae or adults, or all three, depending upon the species. In some species all stages from early spermatogonia or oogonia to the formation of spermatozoa or eggs could be secured from adults, but in most cases it was necessary to use pupae or even larvae in order to obtain the desired stages. This is especially true of the family Drosophilidae. *In all cases the gonads or small bits of tissue were dissected out of the specimens and then fixed;* none of the specimens was fixed entire or partially intact. This fact is emphasized because it has been found that regardless of the fixative used, inferior results are obtained if tissues are fixed in situ.

Dissections were usually made in Ringer's solution except in the case of large specimens, when tissues were dissected out in the body fluid. Dissection in tap water was tried with fairly good results, but mitotic figures were less distinct after this treatment than after the use of Ringer's solution.[2]

For fixation Flemming's strong solution was found most satisfactory and was most frequently employed. Objects were fixed from ten minutes to three hours depending upon their size. Longer treatment was tried, but with less satisfactory results due to frequent osmication and distortion. In addition to Flemming's fluid various other fixatives were tried. Of these Hermann's platino-aceto-osmic, and Gilson's mercuric-nitric gave the best results (in many cases as favorable results as those obtained by the use of Flemming's fluid), especially when it was desirable to differentiate the chromosomes without reference to other nuclear structures. Sublimate acetic and Gilson-Carnoy's acetic alcohol with sublimate were found fairly satisfactory for somatic tissues, but were inferior for the gonads. Bouin's fluid (formol mixture) though frequently used, proved quite undesirable because of its tendency to distort and produce

[2] Dissection in tap water has been recommended by Doncaster ('14) for Abraxis.

clumping of chromatic materials. Good fixation with this method was secured only in the case of eggs and occasional large pieces of somatic tissue where its penetrating power was advantageous.

To supplement the permanent preparations, temporary 'smears' were frequently made with the use of Schneider's Aceto-carmine (Stevens '08 pp. 359–360) which proved to be a valuable agent for rapidly determining whether or not materials contained stages suitable for study. Frequently, one gonad would be prepared in this way and if found to be in the proper stage of development, its mate would be fixed in Flemming. The aceto-carmine preparations often gave very good figures of metaphase chromosome groups, but were found to be unreliable for detailed study because of the frequent distortion incident to swelling or mechanical disturbance. Consequently, most of the observations included within this study are based upon fixed and sectioned material. Sections were made 5 μ thick, except in a very few cases where unusually large cells were found and a greater thickness was desirable. Nearly all slides were stained with Heidenhain's Iron Haematoxylin, either alone or with a counter-stain of eosin or light green. Safranin was used frequently, but gave less distinct images, and failed to differentiate the finer chromatic elements as distinctly as did the haematoxylin.

For the study of cleavage and early embryonic stages Drosophila eggs were used. These were fixed at different periods, from a few minutes to a few hours, after being laid. It was found necessary in most cases to puncture the eggs, in order to facilitate the penetration of the fixative. When the eggs were punctured, successful fixation was secured with Flemming, Gilson's mercuric-nitric, Bouin, sublimate acetic and Gilson-Carnoy, all of which were about equally favorable.

A large proportion of the species included in this study have been reared in the laboratory for one or more generations, and the cytological material which they have furnished has largely been derived from pedigree cultures. In a few instances material was taken from jars of food which had been set out-of-doors, but this was used only when the identification of larvae and

pupae could be determined by the flies which subsequently hatched from the food. In no case is there any question as to the genus of the flies concerned and only in a few cases is the species doubtful. Such cases are mentioned in the text. Of the families Asilidae, Bombyliidae, Syrphidae, Sapromyzidae, Ortalidae and Trypetidae, only adult flies were used.

For the identification of the Sarcophagidae, the writer is indebted to Mr. R. R. Parker, for that of Culex pipiens to Mr. Fred. Knab, for that of the Drosophilidae to Dr. A. H. Sturtevant,[3] and for all other identifications to Mr. C. W. Johnson who has very kindly examined a large series of specimens.

REALITY OF CHROMOSOME PAIRING IN THE DIPTERA

Since Stevens' observations on chromosome pairing in the Diptera were more or less incidental to other features, and since her conclusions have been directly opposed by those of Taylor and of Lomen on Culex—material upon which part of Stevens' work was based—it seems desirable first of all to ascertain definitely whether or not the so-called pairing phenomena in flies do in reality represent the association of independent chromosomes. In the opinion of Taylor ('14) and of Lomen ('14) the duality of the chromatic elements in Culex (and hence by inference in the other Diptera), is due, not to a pairing of two chromosomes but to the precocious splitting of one. Hence they conclude that the haploid number is present in both germinal and somatic cells, and that the somatic divisions are essentially the same as the maturation divisions. According to their idea each chromosome divides in anaphase, giving rise to two daughter chromosomes which remain separated during the resting stage and prophase (thus simulating a pair), and go to opposite poles in the succeeding division.

Before considering the contentions of Taylor and of Lomen further, I will present some of the evidence that has led me to conclude that the double chromatic elements in flies are really

[3] Several species of Drosophila included here are undescribed, and are given Sturtevant's manuscript names.

pairs of chromosomes. This will make clearer the exact points at issue and facilitate subsequent discussion of the contrasting views. The evidence which I wish to present may be considered under three heads as follows:

In the first place the number of chromosome pairs in diploid groups is the same as the number of single chromosomes in mature germ-cells. Figures of the chromosomes in spermatocyte divisions, either first or second, or both, accompany those of diploid groups in most of the species included here, and speak for themselves in this regard. A comparison of figures 13 and 15, 27 and 33, 24 and 25, 44 and 48, 52 and 53, 74 and 77, 125 and 126, 137 and 139, etc., clearly shows the relation between haploid and diploid groups. In some species, the chromosomes are evident even in the spermatids leaving absolutely no doubt as to the number contained in the spermatozoa. It must be concluded, therefore, that fertilization results in a diploid group in which the members of two haploid groups have associated in pairs, unless we resort to the very improbable assumption that an eliminating process intervenes at some stage of fertilization to throw out half of the chromosomes or to fuse them together two by two. Even this assumption, however, is overthrown by the relations of the sex chromosomes described below.

Secondly, if the diploid metaphase group were not made up of pairs, but were composed of double, univalent chromosomes, the two elements of these double chromosomes ought to lie one above the other, not side by side, in polar view, and in early anaphase a haploid group should be seen going to either pole. As a matter of fact neither of these conditions is realized outside of the maturation divisions. The two members of a chromosome pair lie side by side in metaphase, as shown by the figures, except for an occasional displacement, and frequently all of the chromosomes (the double number), may be seen dividing (figs. 7, 8, 9, 16, 28, 32, 40, 77). The side by side association and the method of division are clearly shown in figures 1–5, 7–9, 17, 19–24, 37, 39–46, 77, 98 and 99, etc. Figure 1, for instance, is composed of five symmetrical pairs, the members of which lie side by side. Figure 2 from the same species, shows similar

features. Likewise in figure 3 the side by side arrangement is obvious. Figures 4 and 5, 17, 19 and 20, from species possessing another type of chromosome group, bring out the same relations. In each case the two members of a pair lie side by side, not one above the other—with the exception of one misplaced chromosome in figure 19. Similarly in figures 21–24, representing another type of group, the side by side pairing is very distinct. Other examples are given in figures 27, 28, 37, 39–46, etc. These figures are not selected from among many in which pairing is less evident, but are perfectly typical and represent the normal condition in their respective species.

The manner in which division takes place during late metaphase or early anaphase is shown by figures 7, 8, 9, 16, 28, 32, 40, etc. Figures 7, 8, and 9 represent the same type of chromosome group as do figures 4, 5, 17, 19, 20, namely, a group composed of two long U-shaped pairs, one straight pair, and one small spherical pair. In all of these figures each chromosome (save the smallest in 8 and 9) may be seen dividing equationally, in the ordinary manner. In figure 16 the mode of division in a similar group is seen at a somewhat later stage. The dark chromosomes are seen at a high focus, the light ones at a lower focus. It is evident that each member of the diploid group has divided and sent a daughter half toward either pole. The smallest pair cannot be seen in this figure. In figures 28, 32 and 40 the same process is indicated in the case of two other species. Earlier stages in the same species are represented in figures 27 and 39 respectively. The features indicated by figure 28 are brought out even more clearly by figure 32 (a side view at the same stage). In figure 32 each of the short chromosomes has divided, while the two long ones have split in preparation for division.

Passing now to the later anaphases it may be seen that during this period a diploid, not a haploid, group goes to each pole, and in many cases the two members of a pair of chromosomes are so clearly separated from one another that they cannot be considered the result of a precocious split as suggested by Taylor and by Lomen. This fact is demonstrated conclusively

in those cases in which the two members of a pair have become separated and do not lie side by side in metaphase. A few cases have been found in which the two members of a pair lie on opposite sides of the spindle. In anaphase, each of these is seen to have divided and sent a daughter half to either pole. Figure 29 (same species as 27 and 28), for instance, shows a metaphase in which the two large members lie on opposite sides of the groups. In figure 30 a similarly arranged group is seen in anaphase. It is perfectly clear from the position of the large chromosomes in figure 30 that the two large elements going to one pole are not sister halves of one chromosome, but are daughter halves of two separate chromosomes, else they could not lie on opposite sides of the spindle at this stage. A comparison with figures 27 and 31 shows how this differs from the normal condition in which the large as well as the small chromosomes are paired. The duality of the chromosomes in figure 31, if this figure were taken by itself, might be interpreted as indicating a precocious division of single chromosomes, rather than as indicating pairs of chromosomes, but other facts, as just described, preclude such an explanation. It is doubtless such appearances as those given by figure 31 that have led some authors to misinterpret entirely the nature of Diptera chromosomes.

Fully as convincing evidence is furnished by other cases in which the two members of a pair have become only slightly displaced, instead of lying on opposite sides of the spindle. Such cases are shown in figures 7, 9, 12, 16, 28 and others. Figures 7, 9, 12 and 16 are different stages in nuclei containing the same type of chromosome group. It is obvious that here one of the large pairs has been disturbed in such a manner that its two members resemble two horse-shoes placed side by side. According to the ideas of Taylor and of Lomen these two members should go to opposite poles, but it is clear that they do not. On the contrary each divides and sends a daughter half to either pole. Figure 12 represents a particularly interesting case, for here the chromosomes have all divided and the daughter halves have separated. The figure on the left represents the upper group, that on the right the lower group (displaced in order to

show the chromosomes clearly). Above them is a diagram showing the two groups in position as they appear in the section. Each chromosome in the one group is seen to be represented by a corresponding sister chromosome similarly oriented in the other. Such cases furnish unequivocal evidence that the two members of a pair are not daughter halves of a univalent prophase element, but are distinct chromosomes, and that they both divide equationally in metaphase.

In the third place, diploid groups in the males of species having an unequal X-Y pair, demonstrate by the morphological difference between X and Y that the pair is composed of two distinct chromosomes. A striking example of this is seen in the three species of Drosophila shown in figures 41, 42, 44 and 45 (compare with figs. 49 and 50) in which species the X-chromosome of the males is fully twice the size of its mate Y. It would be difficult indeed to imagine these being daughter halves of a univalent chromosome. The same features are also brought out by other species having unequal sex-chromosomes (figs. 85, 86, 88, 124, 135, 137, etc.), although the evidence is not always so striking as in the three species cited.

These lines of evidence, I believe, leave no escape from the conclusion that pairing of chromosomes is a reality in the species here considered. That the mosquitoes are no exception to this rule will be shown below when the different groups of flies are treated independently.

The essential difference between the above results and those of Taylor and of Lomen center around one particular feature—the behavior of the chromosomes in late metaphase and early anaphase. The other stages are not seriously disputed. The question, therefore, is whether the two metaphase elements separate from one another in anaphase, thus effecting a reduction division, as described by Taylor and by Lomen, or whether each divides and sends a daughter half to either pole as Stevens maintained. I believe that I have demonstrated the correctness of the latter conclusion in the above paragraphs, and need not dwell further on it. The difficulty in the work of Taylor and Lomen is due, I believe, to faulty fixation of their material.

In my experience good preparations have been obtained only when the gonads or small bits of tissue were dissected out and fixed separately—never when the whole insect, or a considerable part of it was fixed intact. The latter method, which is apparently the one used by Taylor and by Lomen, produces a clumping or running together of the chromosomes, which is exactly the kind of behavior that would cause pairs to give the appearance of single chromosomes. Any tendency toward fusion is especially apt to exhibit itself in the anaphases, and hence it is to be expected that such figures as those obtained by Taylor and by Lomen would result whenever the fixation was defective. I have frequently obtained such a result when the fixation was poor, especially after Bouin's, Gilson-Carnoy's or alcohol-acetic fixatives.

DETAILS OF CHROMOSOME BEHAVIOR DURING ONE CELL-GENERATION

The mutual relationship of homologous chromosomes during the various stages of cell division has been carefully studied in both somatic and early germinal tissues of several species, and it is believed that the main facts regarding this relationship are now evident. In brief they are these: In metaphase, either in somatic cells, oogonia or spermatogonia, the chromosomes lie in a flat equatorial plate, the two members of each pair, with occasional exceptions, being arranged side by side as described above (figs. 1, 2, 3, 17, 19, 20, etc.) Each of these chromosomes splits longitudinally, and during anaphase sends a daughter half to either pole, still associated with its mate from the other member of the pair. Figures have already been given (7, 8, 9, 16, 28, 40) showing the chromosomes in the act of splitting, or the daughter halves in the act of separating from one another, also figures (12, 30, 31, 95, etc.) showing later stages in which the halves have become well separated and are going toward their respective poles. Retention of the paired association during anaphase is evident in all, except those in which one or two pairs have been disarranged. In the telophase, the chromosomes become closely massed and rapidly lose their

staining capacity, so that very little can be determined about the behavior of individual chromosomes. It is significant, however, that these chromosomes normally enter the telophase in a closely paired condition (figs. 31, 95, 169, 171) and it seems highly probable that they retain this relationship during the transformations in the resting nucleus. Such a conclusion is rendered almost certain by their subsequent behavior in coming out of the resting stage. The earliest prophase or spireme stages in which the chromatic threads may be distinguished with any degree of clearness show these threads to be intimately associated in pairs (figs. 11, 14, 34, 58, 65, 70, 71, 78, 80, 91, 92, 100, 123, 130, 131, 155, 165); and from this time on they may be seen to retain this association during their condensation and contraction from early prophase up to the time at which definite chromosomes are formed ready to go on the spindle. Some of the earliest prophases in which the chromatic threads were well defined are shown in figures 58 to 63 (Calliphora). Each of the double threads in these figures represents a pair of chromosomes. In figure 62 all six pairs are shown (the smallest being very faint), but in the others only parts of the nucleus are represented. Figure 65 is a later stage showing the chromosomes more condensed and contracted, but still closely apposed in pairs. Figure 66 is a still later stage, in which the chromosomes are assuming their definite shape preparatory to disjoining and going on the spindle. It is followed by the late prophase and metaphase stages represented in figures 53, 54, 55, 56 and 57. These are succeeded in turn by the late metaphase and anaphase in which each of the twelve chromosomes divides equationally as described above. Other early prophases are shown in figures 100 to 102 (Homalomya). The chromosome group here is indistinguishable from that of Calliphora (five large and one small pairs). In figure 100 the long, delicate but double threads are clearly distinguishable. It is impossible to determine precisely how many double threads are present, for some are broken, but the number is clearly about five or six, certainly not ten or twelve. Part of a similar nucleus is shown in figure 101. One of the most interesting features about

these figures (100, 101) is the evident polarization of the chromatic threads. This appears to be characteristic of very early prophases, although such stages are seldom clear enough to draw. When this polarity is compared with that shown by telophases (figures 111 and 112), it is difficult to avoid the conclusion that the two are correlated,—that is, that the chromosomes reappear during prophase in the same relative position, and polarized in the same manner as in telophase. Prophases in other species similar to those cited above are represented by figures 70, 71, and 72 (Musca), 106–108 (Fucellia), 78–80 (Phormia), 91, 92 (Sarcophaga), 130, 131 (Anthrax lateralis) and 123 (Eristalis). These are all essentially alike and involve corresponding chromosome groups. Prophases, together with metaphases for comparison, in species having fewer chromosomes, are shown in figures 14 and 15; 11 and 4, 5; 34, 35, 36, and 4, 5; and 165 and 166.

As seen in the figures all stages subsequent to the condensation of the chromatic elements in early prophase are easily followed, although the behavior of the chromosomes differs slightly in different cases. Usually the association of the two members of a pair becomes loose long before contraction is completed. At this time the two threads are loosely and irregularly coiled about one another (figs. 34, 59, 71, 92), and as contraction proceeds they become more and more loosely associated (figs. 35, 72, 93, 94). Occasionally, however, a close association is retained up to a very late period of contraction (figs. 36, 66, 108, etc.), with the consequent production of figures which very closely simulate those of haploid groups. Such figures as these might readily create the impression of haploid groups in diploid nuclei. By the time spindle formation takes place the chromosomes are usually distinctly disjoined from their mates, although the paired association is still conspicuous and may be very close (figs. 15, 41, 68, 117, 132, etc.) Occasionally the process of separation has been carried on so far that pairing is very indefinite (figs. 153, 161), but such cases are decidedly exceptional. Soon after the chromosomes become arranged on the spindle they begin to show evidences of splitting in preparation for

division (figs. 8, 28, 40, 77, 99, etc.), and by the time the equatorial arrangement is completed they may all exhibit a longitudinal split. It is this stage that demonstrates unquestionably the presence of a diploid instead of a haploid group.

As shown by the figures, especially numbers 7, 28, 29, 68, it occasionally happens, as mentioned above, that the members of a pair appear in metaphase on opposite sides of the spindle, or separated from one another by other chromosomes. This disarrangement apparently takes place in late prophase while the chromosomes are becoming equatorially oriented. Several cases have been observed in which the members of a pair were partially separated by other chromosomes. and it seemed a question as to whether they would be forced completely apart, or would succeed in taking their places together. The frequent appearance of the condition in which the two members are on opposite sides of the plate appears to be due to their having approached the equator of the spindle vertically instead of horizontally, i.e., from one pole instead of from the side—and thus having been pulled diametrically apart, with their points of attachment near together but their extremities pointing in opposite directions. At first sight it would appear that chromosomes once separated in this manner would have difficulty in associating again, and that after many divisions all the pairs would be disarranged. An examination of chromosome arrangement in late anaphase indicates one reason at least why such a confusion does not occur. During this stage the chromosomes are drawn out in a slender cone with their apices brought close together at the pole. As a result all of the chromosomes are rather closely approximated throughout their lengths, and an ample opportunity is afforded for the reunion of separated members of a pair, even if they previously lay on opposite sides of the spindle.

In my paper on Drosophila chromosomes (Metz '14, p. 56), I mention the apparent occurrence of a 'second conjugation' of chromosomes in early metaphase, after the separation which normally occurs in prophase. The details of this phenomenon were obscure at the time, and were left for further study. It

appears now, after a careful study of these stages in a large number of flies, that the so-called 'second conjugation' is of only occasional occurrence, and is not a uniform stage in the chromosomal activities. In some, if not all cases, it is simply a retention of the close approximation that existed in prophase.

When considered step by step, as has just been done, it is remarkable what a resemblance the above processes bear to those of maturation. In the early prophase stages of either somatic or gonial nuclei an almost exact simulacrum of diplotene nuclei is often found. This extends in some cases, even to definite polarization of threads within the nucleus, such as is shown in figures 100 and 101.[4]

PAIRING IN DIFFERENT TISSUES AND DURING DIFFERENT STAGES IN ONTOGENY

No attempt has been made in this study to examine in detail all of the somatic tissues in any one species. Various tissues have been dissected out at different times, however, and fixed with the gonads. In this manner I have been able to study division figures in most of the tissues of the body and during most stages of ontogeny. Among the organs and tissues definitely identified in these studies the following may be mentioned; embryonic brain, eyes, malpighian tubules and wing buds, and somatic as well as germinal parts of the testes and ovaries. I have also examined various bits of tissue taken at random from dissected larvae and pupae of various ages.

In addition to studying isolated pieces, I have studied sections of entire embryos (larvae) in which all of the tissues could be examined. Of course division figures were never visible in all the tissues of these total preparations, but they were frequently found in several parts of one object.[5]

In regard to the ontogenetic development I may state that I have examined all stages from the newly hatched larvae up

[4] See concluding paragraph, page 257.

[5] As mentioned under 'Methods' the figures in total preparations are poor, but they are sufficient to show whether the chromosomes are paired or single.

to the sexually mature fly in several species of Drosophilidae, Muscidae and Anthomyidae.

The results of all these studies on somatic tissues may be summed up in one sentence, namely, that in all tissues of the body and during all stages in development from the newly hatched larva to the adult fly the paired association of corresponding chromosomes is a universal characteristic. So far as I have been able to determine, the pairing phenomena are identical in all diploid cells, whether somatic, spermatogonial or oogonial, from the egg to the adult.

DIFFERENT SPECIES AND FAMILIES COMPARED

In order to determine whether the paired association of chromosomes is characteristic of all Diptera or whether it is restricted to certain individuals or groups, an attempt has been made to study representatives of all the principal divisions in the order. As a result, sixteen families ranging from among the lowest to the highest have been included in the survey. Some of these families are represented by one or two species, others by several species. Since the principal aspects of the pairing phenomena are essentially the same in all of the flies studied no attempt will be made to treat each individual species. Instead, a few characteristic members will be chosen as representatives of the respective families. Likewise, no attempt will be made to give a complete account of the chromosome behavior in each species treated. In many cases only enough figures are reproduced to show the nature of the chromosomes and their paired association.

For convenience the order of treatment of the families is the reverse of that given in the synopsis (i.e., from the highest to lowest instead of vice versa), except that the Muscidae will be considered before the Sarcophagidae.

Muscidae

Calliphora erythrocephala (figs. 51–66). Figures 51 and 52 represent the haploid group of this species, taken from first spermatocyte divisions. The group consists of four similar,

long chromosomes, one shorter chromosome and one small, spherical chromosome. Figures 53 to 57 illustrate corresponding diploid groups of the same species taken from ovarian (53–56) and somatic (57) cells. From these figures it is evident that for each single chromosome of the haploid group there is a pair of chromosomes in the diploid group, and that the members of this pair are in close proximity to one another. Earlier stages, showing the origin of the pairs in prophase, are given in figures 58–66. Some of the figures represent only sections of the nucleus, but others (58, 62, 63, 65, 66) are taken from uncut nuclei and include all of the chromatic material. In early prophase stages the five pairs of long chromosomes are clearly represented by the five long, double threads as shown in figures 58, 62 and 65. Frequently the small pair is concealed and cannot be distinguished, but in many cases it is as clearly evident as are the others (figs. 62, 65). The duality of the threads in early prophase is perfectly distinct in almost all cases. The figures given here are entirely typical of scores studied, and are taken from various tissues of the body all of which show the same phenomena in dividing cells. Very rarely a figure is found in which no duality can be seen in the threads (fig. 63), but it seems certain that this appearance is due merely to overstaining which conceals the true dual nature. Figure 65 is a good example of such a case. When first studied the members of this group appeared to be perfectly homogeneous elements and were drawn as such, but after the material had cleared in balsam a few months, the duality of the threads became very evident, as shown in the figure. I have no hesitancy, therefore, in considering figure 63 to be of the same nature, especially since it is almost the only clear case of its kind found.

During later prophase stages such as shown in figures 53, 55 and 66, the chromosomes rapidly contract, and condense, and the members of a pair dissociate somewhat in preparation for division. When they go on the spindle they form a flat equatorial plate, with corresponding chromosomes arranged side by side in the same plane. Only in exceptional cases, such as are in-

evitable under the circumstances, are the two members of a pair in any other relation than this during metaphase.

Musca domestica (figs. 68–72). In Musca the chromosomes are very similar in form and behavior to those of Calliphora, except in respect to the sex-chromosome pair, which is almost as large as the autosomes. Haploid groups of Musca have already been published by Stevens ('08, fig. 3). The accompanying figures are taking solely from diploid groups to illustrate the pairing phenomena. They are all from ovarian tissue far in advance of maturation stages and may be said to represent the characteristic features of prophase and metaphase in early ovarian and somatic cells. Figures 68 and 69 are metaphases showing the six pairs of chromosomes in the equatorial plate. It will be noticed that in each figure the members of one pair of chromosomes are displaced and are not closely associated. These are in all probability the sex-chromosomes (XX). Prophases showing the early appearance and the disjunction of the chromosomes are represented in figures 70, 71 and 72. The former is from an entire, or nearly entire nucleus, the latter two are from cut nuclei, but each includes almost all of the chromatin.

Phormia regina (figs. 73–80). Haploid groups of this species are shown in figures 73 to 75 (second spermatocytes) and figure 76 (first spermatocyte). As shown in figures 73 and 75 in contrast to 74, the sex-chromosomes (smallest in each case), are very unequal. In figure 76 they may be seen separating from one another in the reduction division. Figure 77 is taken from a spermatogonial cell in early anaphase (or late metaphase), and shows the six pairs of chromosomes, corresponding to the six single chromosomes of the haploid group; each of these is split lengthwise in the process of division. In the center may be seen the unequal X-Y pair splitting in the same manner as are the autosomes. A comparison of this figure (77) with that of a similar stage in the reduction division (76) clearly brings out the relation between the two groups (haploid and diploid). Prophases from early ovarian tissue showing the origin and behavior of the pairs in preparation for division in

diploid nuclei are given in figures 78 to 80. They differ in no essential respect from those in Calliphora and Musca.

Likewise the other Muscidae studied (Muscina stabulans, Calliphora viridescens, Lucilia sericata, and Pseudopyrellia sp.) agree with those already described.

Sarcophagidae

Sarcophaga (figs. 81–97). Several species of Sarcophaga have been used in this study and have been found to agree so completely in respect to chromosome behavior that they will be treated as a whole. For specific references see explanation of figures on p. 270. Haploid groups from second spermatocyte divisions are given in figures 81 and 82, and from first spermatocyte divisions in figures 83 and 84. The last named is a side view showing the inequality of the X Y chromosomes at the time when they separate during reduction. Corresponding diploid groups are represented by figures 85–88 (spermatogonial), figure 89 (ovarian follicle cell) and 90 (somatic, embryonic cell). In the male groups (figs. 85–88) the difference between X and Y (smallest chromosomes) is plainly evident. Prophases showing the early appearance of the pairs, and quite comparable with those in the Muscidae, are given in figures 91 (somatic, two sections of same nucleus), and 92 to 94 (somatic). An anaphase from a similar cell (embryonic glandular tissue) is given in figure 95. It clearly shows the persistence of the paired association and indicates the relative positions occupied by chromosomes when they enter the telophase and subsequent resting stage. In this figure the spindle fibers are schematized, but the chromosomes as in other figures are drawn in their exact position. Figures 96 and 97 are taken from multiple groups (somatic) showing respectively 24 and 48 chromosomes. The former is significant because it shows tetrad aggregates instead of pairs (compare with figs. 85–90 and see pp. 252 and 253). In the latter the chromosomes are so massed together as to obliterate the associations.

Ravinia peniculata (figs. 98, 99). This species is indistinguishable from those of Sarcophaga in respect to pairing phenomena. Figures 98 and 99 are ovarian (early pupal) metaphases showing the six pairs of chromosomes essentially like those of Sarcophaga. The latter shows the metaphase splitting of the chromosomes very clearly (compare with figure 77).

Anthomyidae

Homalomya sp. (figs. 100–105). Particularly clear prophase figures have been secured in this species, both with respect to somatic and to spermatocyte divisions. The chromosome group is practically indistinguishable from that of Calliphora (figs. 51–57). Figures 100 and 101 are very early prophases from somatic nuclei, illustrating the configuration of the chromatic threads at this time. The former is from an entire, or almost entire nucleus, in which the bivalent (double), long drawn out threads, each representing a pair of chromosomes, are discernible. Attention is particularly called to the polarization of these threads and the resulting similarity in appearance between this somatic prophase and the synaptic stages accompanying maturation in many other animals. Figure 101 represents a similar stage from the same tissue, but includes only a portion of the nucleus. A later stage in which these threads lose their polarity and contract before giving rise to the metaphase chromosome pairs is shown in figure 102. In comparison with such somatic and spermatogonial prophases it is of interest to examine corresponding stages in the maturation divisions. Figure 103 is a portion of a second spermatocyte prophase and shows sister chromosomes closely intertwined preparatory to going on the spindle. In metaphase (fig. 104) they come to lie one above the other in the equatorial plane. Figure 105 is a second spermatocyte anaphase. In figure 103 only three of the chromosomes are represented, but in 104 and 105 the full (haploid) complement is present. The double elements in these cases are split univalten chromosomes, the two members of which separate in anaphase as shown in figures 104 and 105. It is important

to note that at certain stages in prophase the figures of all three (somatic, first maturation and second maturation) divisions are superficially very similar, although the actual processes in the three cases are very different.

Fucellia marina (figs. 106–110). As in the previous case, so in the present, the paired relationship of the chromosomes is essentially like that described for the Muscidae and Sarcophagidae, and requires no detailed description. A few somatic prophases have been reproduced to show the origin of the chromosomes in the former in the form of closely paired threads, and the subsequent disjunction of these into the less closely associated condensed chromosomes found in metaphase. Figure 106 is an early prophase showing the six bivalent threads. Figures 107 and 108 are somewhat later stages illustrating the separation of the threads. All three are complete (diploid) figures. The most interesting features observed in Fucellia are those shown by prophases containing multiple (probably tetraploid) groups (figs. 109, 110). Each chromatic aggregate in these, contains four (or eight) chromosomes instead of the usual pair, (compare with figs. 96 and 97 and see pp. 252 and 253).

Ophyra leucostoma (figs. 111–114). In most of the Diptera studied so far great difficulty has been experienced in analysing telophase figures. Usually the chromatin is so massed at this point that no details whatever can be distinguished. In the present species, however, a few figures have been obtained, which although far from satisfactory, are nevertheless sufficient to show something of the chromosomal behavior during this stage. Two of these are shown in figures 111 and 112. They suffice to show the loop or U-shape of the chromosomes, and suggest the process of reticulation that is taking place as the chromatin becomes diffuse. The polarity of these U-shaped threads bears a significant relation to the similar polarity evident in early prophase when the chromosomes reappear (figs. 100 and 101). The chromosome group and the pairing phenomena of Ophyra are practically the same as those of Homalomya and Fucellia. Figure 113 shows a late diploid (spermatogonial) prophase with six pairs of chromosomes, some of which already

indicate the metaphase split; and figure 114 shows a corresponding but somewhat later stage in the first maturation (reduction) division.

Sciomyzidae

Neuroctena analis (figs. 115, 116). There is nothing peculiar about the chromosomal behavior in the Sciomyzidae, so far as I have been able to determine. Several specimens of N. analis have been studied, with results comparable in every way to those already described. The two accompanying figures are sufficient to show the paired association and the relation between haploid (fig. 115, second spermatocyte) and diploid (fig. 116 spermatogonial) groups.

Trypetidae

Flies of this family, so far as my experience goes, are not favorable for chromosome studies. Nevertheless they present sufficiently clear figures to show that the paired association is present here just as it is in other flies. Most of my studies were made upon Euaresta melanogaster, material of which I secured in Cuba. The chromosome group of this species appears to be composed of six pairs similar to those in the Muscidae, although no figures have been found that are complete and at the same time clear enough to settle this point.

Ortalidae

No embryonic stages (larvae or pupae) have been secured from any members of this family, and consequently no somatic divisions have been studied. Spermatogonial and spermatocyte divisions have necessarily formed the basis of my observations on both of the following species, yet there can scarcely be any question that there is a definite correspondence between the phenomena exhibited by spermatogonia and somatic cells.

Chaetopsis fulvifrons (figs. 117–119). Chromosomal behavior in spermatogonia of this species corresponds fully with that described for ovarian and somatic cells in species of Drosophili-

dae (figs. 4–20) having a similar chromosome group. In Chaetopsis no good figures of early spermatogonial prophases have been secured, owing to the small size of the nuclei, and to difficulties in fixation. Metaphases, however, are distinct (figs. 117–118) and plainly show the paired arrangement of the chromosomes. These, when compared with maturation divisions showing the haploid group (fig. 119, first division) leave no doubt of the relations in this species.

Camptoneura picta (figs. 120, 121). Since C. picta shows pairing relations similar to those in the last named species it attracts attention only because it differs so markedly from Chaetopsis in respect to the number and size relations of its chromosomes. As a matter of fact Chaetopsis excites the greater interest, for Camptoneura has the chromosome group (fig. 120, diploid, and 121, haploid) found in several families (all those above mentioned, as well as the Sapromyzidae, Micropezidae, Sepsidae, Syrphidae, and one species of Bombyliidae), while the group found in Chaetopsis is found in no other species I have studied outside the Drosophilidae.

Sapromyzidae

Physegenua vittata (fig. 122). I have had difficulty in obtaining suitable material from Sapromyzid flies, but as in the case of the Trypetidae enough has been secured to determine the essential point—that the chromosomes are associated in pairs. Figure 122 (spermatogonium) represents one of the few complete polar views found. It is seen somewhat diagonally, with the result that some of the pairs appear to lie beneath the others, but in reality they form an almost flat plate, entirely comparable with those seen in the Muscidae, etc. The two small chromosomes are doubtless the sex-chromosomes (X Y), just as are the small ones in the Muscidae.

Drosophilidae

(See pp. 222–224, "Reality of chromosome pairing." For specific references see explanation of plates; also Metz '14.)

Syrphidae

Eristalis tenax. My studies in this species have included pupae as well as adults, and in both I have found the chromosome behavior to agree with that in the cases described above, and with Stevens' ('08) description.

Eristalis bastardi (fig. 123); *Volucella obesa* (figs. 124–126); *Mesogramma marginata* (figs. 127, 128). These three species are very different from one another in appearance, but their chromosomes appear very similar (save for minor details of size relations) and hence will be considered together. Figure 123 (Eristalis bastardi) represents part of a prophase figure showing the bivalent chromatic threads which are comparable in every way with those seen in Homalomya, Sarcophage, etc. Figures 124 and 125 are metaphases (spermatogonial) of Volucella, and clearly show the paired relationship. In the former one chromosome is missing, leaving a single member (in left margin of group) without a mate, but otherwise all are paired. This species is particularly interesting because of the different sizes apparent in its chromosomes. One pair is easily recognized by its large, and one (sex-chromosome) by its small size, and even the others show slight differences from one another. Figure 126 is a first spermatocyte division for comparison with the diploid groups; note the unequal X and Y chromosomes, which are paired in the diploid groups. Figures 127 and 128 (spermatogonial) of M. marginata are of significance only in showing the paired arrangement of the chromosomes.

Sepsidae

Piophila casei. There is no marked distinction between P. casei and the various species of Muscidae and Sarcophagidae, either in chromosome numbers and size relations or in the general chromosome behavior.

Bombyliidae

Anthrax lateralis (figs. 129–133). No more conspicuous cases of chromosome pairing have come to my attention than those

exhibited by this and other species of Bombyliidae. Figures 129 to 133 are only a few from among scores of similar ones studied. In all cases the five large pairs and often the small pair stand out clearly and show a close approximation. The figures need little explanation beyond that given already for preceding species. Numbers 129 to 131 are spermatogonial prophases showing the five long and one short double threads, which later loosen up and contract to form the metaphase pairs shown in figures 132, 133.

Anthrax sinuosa (figs. 134–140). This species is very interesting from several standpoints. In the first place it possesses chromosome pairs of various sizes (figs. 134–137), which clearly illustrate the pairing of corresponding chromosomes. Secondly the evident dissimilarity between A. sinuosa and A. lateralis in number of chromosomes, the former having 18, the largest group in any fly within my knowledge, and the latter possessing but twelve, presents the greatest divergence of this nature that I have observed between two species in one genus. Thirdly, the sex-chromosome pair is apparently one of the largest in the group, instead of the smallest, as has been the case in all of the above species exhibiting a conspicuous inequality between X and Y. Unfortunately I have been unable to identify the sex-chromosome pair in A. lateralis. If the small pair in A. lateralis (figs. 132, 133) is the sex-chromosome pair, as it is in many flies, then a remarkable difference exists between the sex-chromosomes of the two species, such a difference as I have found in no other closely related flies. Similar differences have been observed between related species of Hemiptera and Coleoptera, but seem to occur very rarely among the Diptera. In maturation divisions of this species (figs. 138–140) the short chromosomes show a tendency to become rounded, but the relative sizes are readily seen to correspond with those of the diploid groups. Figures 139 and 140 (second spermatocytes) appear to be respectively X- and Y- containing groups. As the spermatogonial figures (134–137) show, X is the largest chromosome present, while Y is smaller than the two largest autosome sizes. Comparing figures 139 and 140 it may be seen

that the latter contains three large chromosomes (X and the two largest sized autosomes), while the former (139) has only two large chromosomes but has an extra small member which must be Y. No sufficiently clear first maturation divisions have been found to show the X-Y relations of that stage, unless the apparently single element projecting from the largest chromosome in figure 138 is the unmatched end of X. If so, one of the smaller pairs is concealed. The figure is drawn just as it appears, but I am not sure of its significance.

Spogostylum simson (figs. 141, 142). No males of this species were secured, but very clear figures were observed in ovarian follicle cells. Two of these are given to indicate the similarity between the pairing here and in the other species. Figure 141 is a metaphase plate showing the diploid group and the association in pairs. Figure 142 illustrates a similar cell in prophase with corresponding chromosomes forming closely united double threads in the characteristic manner. As the figures indicate, this group differs markedly from both species of Anthrax in the size and form relations of its members. Apparently there is no dominating type of chromosome group in the Bombyliidae such as is seen in the majority of other families.

Asilidae

Twelve species of this family have been studied as indicated in the synopsis (p. 217), but only a few of them need be considered. Those chosen are selected particularly to illustrate the various numbers and sizes of the autosomes, and the varying degrees of inequality of the sex-chromosomes. Pairing is constant in all of them.

Asilus sericeus (figs. 143–145). This species has perhaps the most simple group found in the family, containing as it does only five pairs of chromosomes, and lacking any conspicuous inequality between the sex-chromosomes. Yet it is one of the most interesting groups I have found, for each pair appears to differ from all the rest in respect to size. The two large pairs are admittedly

very nearly the same size, but even they may be distinguished in some figures (note especially figures 143 and 144).

Asilus lecythus (figs. 146–148). Scarcely less striking in the matter of size differences is the evidence presented by this species. Upon close examination its seven pairs (or its seven single chromosomes in haploid groups) are seen to be definitely graduated in size from the smallest to the largest. The gradations are somewhat confused in the diploid groups by the unevenness and the flexures of some of the chromosomes, but in haploid groups (fig. 148, second division) the gradation is much more conspicuous. The sex-chromosomes, apparently, are not unequal.

Asilus notatus (figs. 149, 150). What has been said of the last species (A. lecythus) applies equally to the present one, except that the size differences between the larger pairs are scarcely distinguishable. Figures 149, 150 show spermatogonial and second spermatocyte groups of this species.

Leptogaster badius (figs. 151, 152). The diploid group of this species is shown in figure 151. As may be seen it consists of five pairs, only two of which may be differentiated by size. The largest of these is the sex-chromosome pair, whose members, as in previous cases are frequently not associated during metaphase. The haploid group is indicated by figure 152 (second division).

Erax rufibarbis (figs. 153, 154). In this species, also, five rather similar pairs of chromosomes are found. As in the previous case only the smallest and largest (sex chromosome pair) may be differentiated. Figure 153 shows the chromosomes in a flat plate and indicates their size relations. In spermatocyte divisions the chromosomes of E. rufibarbis show a decided tendency to condense and become rounded, but the size relations are nevertheless conspicuous (fig. 154). This tendency toward condensation extends even into the spermatids, thus enabling one to count the chromosomes with ease, and to determine without doubt the number of chromosomes carried by the spermatozoan into the egg.

Dasyllis thoracica (figs. 155–158). D. thoracica furnishes evidence very similar to that presented by Asilus sericeus. No two of its five pairs of chromosomes (fig. 156) appear to be the same size. The smallest and next smallest pairs are very distinct, as is also the largest. Possible confusion arises then, only in connection with the two intermediate pairs, but since one of these appears to be the X-Y pair its dimorphism, if the apparent dimorphism is real, serves to differentiate it from the other intermediate pair. I have been unable to obtain sufficient spermatogonial figures to determine definitely the sex-chromosome relations, but evidence from the first spermatocyte divisions makes it probable that the relations shown in figure 156 are correct. In the first spermatocytes (fig. 157), one of the intermediate pairs (corresponding to XY in figure 156) appears to have a univalent attachment (X in the figures) at one end, which strongly suggests the unpaired end of an X-chromosome. Analysis of the first spermatocyte group (fig. 157) then, reveals one small spherical chromosome (1), one small, elongate chromosome (2), one larger, symmetrical chromosome (3), one similar, but asymmetrical chromosome (4), and one largest chromosome (5), each distinct from all of the others. In the diploid group each of these is represented by a pair of chromosomes. A diploid group showing the intimately paired association in prophase, similar to that in the Muscidae, etc. is given in figure 155. A second spermatocyte, haploid group is shown in figure 158. The seeming duality of the largest chromosome here is simply due to the metaphase split, and is not related to the apparent sex-chromosome dimorphism of the first division.

Deromyia winthemi (figs. 159–164). The six pairs of chromosomes in this species (figs. 159–161) are graduated into four sizes, of which the largest and smallest are represented by one pair each, and the two intermediates by two pairs each. In some figures (161, 164) even these intermediates appear to be individually differentiated, but the distinctions are not great. The sex-chromosomes (X and Y) are very dissimilar, and, as shown by the figures, are more often dissociated than are homol-

ogous autosomes. Figure 162 shows the X and Y -chromosomes separating from one another in the reduction division. Figures 163, 164 are second spermatocyte groups showing the X-containing and Y-containing classes.

Stratiomyidae

Ptecticus trivittatus. No differences in chromosome behavior (so far as the paired association is concerned) have been found to distinguish this species from those previously considered. P. trivittatus possesses eight pairs of chromosomes, of which the smallest is the unequal sex-chromosome pair.

Culicidae

Culex pipiens (figs. 165–171). Since exception has been taken to the observations of Stevens on the chromosomes of Culex pipiens (see p. 221), I have made a careful study of this species in order to determine whether any fundamental differences exist between it and the higher Diptera with regard to chromosome pairing, but I am not able to find such differences. My studies are based upon spermatogonia and ovarian cells from larvae and pupae. In these I find the six chromosomes closely associated in pairs during prophase (fig. 165), dissociating somewhat in late prophase, and arranging themselves side by side in a flat plate during metaphase (figs. 166–168) just as in the other Diptera. There is no evidence whatever, in my material, of a separation (reduction) of the two members of a pair during anaphase such as described by Taylor and by Lomen. On the contrary, anaphase figures clearly show each chromosome dividing and sending daughter halves to the poles. Figure 170 (a portion of an early spermatogonial anaphase in side view) shows the manner in which each individual chromosome divides. Figure 169 shows a later stage of a typical anaphase (spermatogonial) also in side view, in which six chromosomes (three pairs) are each undergoing a division. At this stage the chromosomes are in the form of double V's each of which is a daughter chromosome attached at its apex to a spindle

fiber. In the figure (169) the V-shaped chromosomes are all seen edgewise, so that one arm lies almost directly below the other (indicated by light shading.) The lower arms of the pair on the left are not visible (apparently being cut off by the knife), but the other two pairs are entire and clearly show the method of division. It is perfectly plain that the two chromosomes in the pair on the left have completely divided, that those in the center have almost divided, while those on the right have only partially divided and show the daughter halves attached for some distance at their ends. Figure 171, in which only two of the three pairs are drawn, shows the same features. It is obvious that such figures as these could not possibly result from a division in which the two members of each pair went to opposite poles, even supposing them to split in early anaphase as conceived by Taylor and by Lomen. The figures reproduced here are only a few from among many studied, all of which present the same features.

There can be no question, therefore, that in the ordinary (diploid) mitoses in Culex, the two members of a chromosome pair, lying side by side in the metaphase plate (figs. 166–168), both split longitudinally in the equatorial plane (transversely to the axis) of the spindle, and that each sends a V-shaped daughter half to either pole, or in other words, that an equation division is effected. This is in direct opposition to the ideas of Taylor and of Lomen who concluded that the two members of a pair lie one above the other in metaphase, that they go to opposite poles in anaphase (effecting a reduction division) and that as they go they split in a line parallel to the axis of the spindle. As I have heretofore stated (p. 226) I believe that Taylor's and Lomen's errors are due to poor preparations in which the anaphase chromosomes were so massed together as to entirely conceal their true nature and behavior.

DISCUSSION

One of the most interesting chapters in the history of modern biological progress is that marked by the rise into prominence of the 'chromosome theory' of heredity. And contributory

to the development of this theory probably no single conception has been of more value than that which postulates a qualitative differentiation among the chromosomes (Boveri '01), and an individual homology between respective members of the two gametic groups (Montgomery '01). The growth of this conception is of particular interest in the present connection.[6] It was based, of course, upon the foundation laid by Van Beneden's 'law' ('83) of the equivalence of maternal and paternal chromosome groups, and upon the principles of chromosomal individuality and continuity developed by Rabl ('85), Boveri ('87, '88, '91), Herla ('93), Zoja, Van Beneden and others, but not until 1901 did it assume its present features. From Montgomery first came the idea that each chromosome in the spermatozoon has an equivalent mate in the egg, that fertilization brings the two together in one cell, and that maturation segregates them again into different cells—the gametes.[7] These conclusions were based upon a study of several Hemiptera (Protenor, Peliopelta, Zaitha), in which certain pairs of spermatogonial chromosomes, distinguished by size and shape, apparently became associated in synapsis and underwent segregation in the reduction division. The almost simultaneous and even more far-reaching observations of Boveri ('01) were from his well known experiments on dispermic sea-urchin eggs, in which he demonstrated a qualitative difference between the respective chromosomes in their effect upon development.

Further attention may be confined to features relating to chromosome pairing. The first of these is the discovery by Montgomery in 1904 and 1905 of a paired association of corresponding chromosomes in cells other than those involved in the maturation process. These observations were made upon Plethodon, and upon the Orthopteran, Syrbula, in the latter of which he found twelve of the twenty chromosomes to possess size differences enabling him to assort them into six groups of

[6] For a comprehensive review see Wilson '05, '14, Conklin '14, East '15, and Morgan, Sturtevant, Muller, Bridges '15.

[7] This conclusion was forecasted perhaps by Henking in 1891, and by Montgomery in 1900, but was first given definite expression by Montgomery in 1901.

two each. The members of these groups, according to his observations are already actually associated in symmetrical pairs in the last spermatogonial divisions and later, in the spermatocytes, undergo synapsis and reduction.

After once discovering pairing in spermatogonia he returned to the subject again in 1908 with additional evidence based on studies of Ascaris, and again in 1910 with more evidence on the Hemiptera.

In 1902 Sutton described a significant case (Brachystola) in which he believed that all of the chromosomes could be assorted into pairs according to size characteristics. It should be noted that the chromosomes in Brachystola are not actually arranged in pairs, and that the size differences between them are scarcely sufficient to make possible an accurate analysis; yet in spite of this the probabilities afford strong support to Montgomery's deductions. Further support was given by Janssens and Willems ('08), whose description of paired chromosomes in spermatogonia of Alytes corroborated that of Montgomery on Plethodon. Similarly, the studies of Wilson, Payne and others on the Hemiptera, of McClung and his students on Orthoptera, of Stevens on Coleoptera, and of various others, plainly demonstrated that in animals possessing chromosomes of different sizes and shapes there are always two (or multiples of two), of each kind (excepting the sex-chromosomes of the male).

Contemporaneously with these researches in the field of zoölogy, there was taking place a strikingly similar development along botanical lines. Indeed it is an interesting coincidence that almost simultaneously with Montgomery's discovery of pairing in the spermatogonia of Syrbula, Strasburger ('05) observed a like association in certain plants. He even went one step further than Montgomery in finding the paired association in somatic cells, entirely distinct from the germinal tissues. In embryonic nuclei of Galtonia candicans he found four small and eight large chromosomes, which exhibited an association in pairs. Likewise in Funkia Sieboldiana he observed twelve large and thirty-six small chromosomes showing a similar paired relationship. "Ich habe zu oft in den Geweben von Galtonia,

und noch haüfiger von Funkia in vorgerüchten Prophasen gleich grosse Chromosomen in Paaren nebeneinander liegen sehen . . . . " ('05, p. 19). The pairing in these cases, as in those of Montgomery, is seldom intimate, if one may judge from the published figures, but there can be little question that it is real.[8] Somewhat later paired chromosomes were recorded by Strasburger ('07) in root-tips of Pisum, by Sykes ('08) in Hydrocharis, Lychnis and Bryonia,[9] and by Overton ('09) in root-tips of Calycanthus floridus, where the chromosomes are said to be arranged in pairs not only during metaphase, but also (as prochromosomes) in resting stages and prophases. In Calycanthus, as in Pisum and some of the other cases, the size difference between respective pairs is not noticeable, but the pairing is very intimate, and if Overton's counts are correct there can be no doubt as to the essential facts.[10] In the same year Müller ('09) described a pairing of chromosomes in somatic metaphases of Yucca. The statements of Müller were soon challenged by Bonnet ('11) who maintained that since only two sizes of chromosomes were present, and there were numerous representatives of each, such associations as those described by Müller were probably due merely to chance. In view of Müller's recent work ('12) however, in which he describes unmistakable cases of pairing in other plants, it seems unlikely that he was mislead by purely accidental phenomena in the previous case.

In 1910 Strasburger described further cases of chromosome pairing in root-tips of Melandryum rubrum, Mercurialis annua, and Cannabis sativa, in each of which different sized pairs were evident, although the spatial association was not very conspicuous. Stomps ('10, '11) during the same period found a comparable pairing in Spinacia, a plant possessing three large and three small pairs of chromosomes. Similarly Nemec ('10) work-

[8] Confusion has arisen in some cases by the application of the terms 'pairs,' 'paired chromosomes,' etc. to split, univalent chromosomes, and in other cases by a difference of opinion between different investigators on the same material, but those cited here are all based upon reasonably good evidence.

[9] Sykes at the same time confirmed the observations of Strasburger on Funkia and Pisum.

[10] His conclusions have been disputed by von Schustow '13.

ing on the root-tips of Ricinus, Kuwada ('10) on Oryza sativa, Tahara ('10) on Morus alba and M. indica, and Ishikawa ('11) on Dahlia coronata all observed evidences of pairing in somatic cells. The observations of the three Japanese authors are particularly convincing because of the variety of sizes among the chromosomes with which they deal, and the symmetry of the pairs. Shortly afterward Gates ('12) records slight evidences of pairing in Oenothera and expresses his belief that pairing in somatic metaphases "is widespread in the sporophyte tissue of plants" (p. 1004). During the same year Müller ('12) in a comprehensive study of metaphase pairing in plants figures and describes the paired condition in more than a dozen species, several of which had not been treated previously. Among the species described by Müller the following furnish convincing evidence: Najas marina, Galtonia candicans, Listera ovata, Albuca fastigiata, Aloe Hanburyana, Eucomis bicolor, Bischornerea superba, Bulbine annua, Nerine rosea, Muscari botryodes, Scilla bifolia, Chinodoxa Luciliae, and Hyacinthus orientalis.

It can hardly be said, however, that the conclusions of these various authors have been received by cytologists without criticism or opposition. True, most of the critics have been simply sceptical, rather than openly antagonistic, but others have been radically opposed to some or all of the conclusions. Chief among the critics are Meves, Fick and Della Valle on the one hand, and Dehorne with his adherents on the other. Meves, Fick and Della Valle object to practically the whole chromosome theory (Meves '07, '08, '11, etc.) and hence incidentally to the hypothesis of chromosome pairing. Since it is not in the province of this paper to consider the whole chromosome theory, only the criticisms relevant to pairing will be reviewed. The others have been repeatedly and completely answered by previous authors (Boveri, Strasburger, Gregoire, Wilson, Montgomery, etc.). Meves has presented the arguments of himself Fick and Della Valle relative to chromosome pairing, in connection with a study of Salamandra ('11). As a result of this study, he concluded that the chromosomes can neither be assorted into pairs according to size, nor can they be said to arrange themselves

in pairs through side by side approximation. Upon this basis he decided that the entire hypothesis of chromosome pairing is a delusion. His attitude toward this matter, however, is so obviously biased as to discount very materially his whole argument. Dealing as he does with a chromosome group composed of large, numerous and almost uniform members it is little wonder that he finds no conspicuous evidence of their being differentiated into pairs. The wonder is that he attempts to draw conclusions of any final nature regarding this problem from material so evidently unsuited for its solution. The only answer to be given to Meves argument is that it does not accord with the facts as presented by organisms in which the chromosomes are sufficiently differentiated to be susceptible of analysis. The conclusions of Meves on this question have been directly controverted by von Baehr, Montgomery, Müller ('12), Lundegardh ('13) and others.

The criticism of Dehorne and his adherents is in the nature of an alternate theory, based upon the conclusion that all chromosomes are constantly dual or quadruple in form. Upon this basis 'pairs' of chromosomes are very readily explained as simply being halves of single chromosomes derived from a precocious split. If the two members are themselves split, then the single chromosome is represented by a quadruple element or tetrad. According to this theory each univalent, metaphase chromosome is represented by four parallel elements or two dyads. During anaphase these dyads separate from one another (passing to opposite poles) and then immediately split again to re-form the tetrad. Thus a quadruple structure is maintained throughout the greater part of any cell generation.

Such a theory, if true, would afford a very simple explanation of 'pairing'; but unfortunately it cannot be reconciled with the facts. In the first place Dehorne's evidence is directly contradicted by the actual history of the chromosomes as reexamined by Gregoire and Muckermann; and in addition, as pointed out by these authors and by von Schustow ('13) and von Baehr ('11) it takes no account of the relation between haploid and diploid groups or of the evidence furnished by the

sex-chromosomes, which shows that members of pairs, whether associated together or not, are separate and distinct chromosomes instead of daughter halves of single chromosomes.

The actual behavior of chromosomes in the Diptera shows with the greatest clearness that neither the criticisms of Meves nor of Dehorne can be valid in this group. The evidence leaves no doubt that the chromosomes are arranged in pairs and are paired in accordance with their size and form. In Dasyllis thoracica (figs. 155–158) for instance the five pairs include four sizes, of which the smallest, next smallest and largest are individually distinct. Similar relations are seen to exist in various other species, such as Asilus lecythus (figs. 146–148), Asilus notatus (figs. 149, 150), Deromyia winthemi (figs. 159–164), Neuroctena analis (figs. 115, 116), Volucella obesa (figs. 124–126), Mesogramma marginata (figs. 127, 128), Chaetopsis fulvifrons (figs. 117–119), Anthrax sinuosa (figs. 134–137), Spogostylum simson (fig. 141), Asilus sericeus (see p. 241) and certain species of Drosophilidae (see especially figs. 21–26). When the haploid and diploid groups of any of these species are compared they are seen to contain the same series of sizes, the former having one and the latter two representatives of each size. There can be little doubt, therefore, that each pair in the diploid group is composed of one paternal and one maternal member; indeed it only remains to establish the continuity of the chromosomes to make this a demonstrated fact.

Another question upon which the Diptera present definite evidence is that of gonomery. In contrast to the more or less continued spatial separation of the two parental chromosome groups found (Haecker, Van Beneden, Rückert, Conklin ('02), Blackman, Ferguson, etc.) in some organisms, the parental groups in the flies intermingle, and the corresponding chromosomes become arranged in pairs at an early stage in the cleavage of the egg,—perhaps during fertilization and before the first cleavage, although this has not been observed. The earliest stages which I have been able to study with accuracy are those immediately following the migration of the cleavage nuclei to the surface of the egg; and these show the chromosomes

definitely paired. A late prophase group from one of these nuclei is shown in figure 47 (note the association of X and Y). Subsequently to this stage pairing remains constant throughout the development of the fly.[11]

As to the causes of chromosome pairing in the Diptera very little may positively be said, but there are certain facts about the phenomena which should be considered in this connection. The facts indicate for instance, that pairing is not due to purely mechanical causes, but is dependent in some way upon the qualitative nature of the chromosomes. This conclusion seems evident from the fact that paired chromosomes are corresponding or similar chromosomes. It is difficult to conceive how purely mechanical forces can cause anything more than random pairing, while as a matter of fact the actual pairing is selective to the highest degree. That this association is not merely an assortment according to size is shown by the pairing of unequal sex-chromosomes in the males (figs. 41, 42, 44, 45, 86, 88, etc.), where X is often several times as large as Y.

A suggestion as to the significance of pairing may be obtained from tetraploid groups such as are found occasionally in embryonic somatic tissues.[12] One such is shown in figure 96. In this case there is twice the normal number of chromosomes (24 instead of 12), which means that in place of two chromosomes of each kind, there are four. On the assumption that homologous chromosomes associate together, these 24 chromosomes ought to associate in groups of four; and this is actually their arrangement. In figure 97 is shown a multiple group containing four times the normal chromosome number, or 48. In this case the chromosomes are so crowded together that their grouping is confused, and it is impossible to tell how they are associated. In prophase nuclei of a similar kind, however, the association of homologous chromosomes is clearly evident. Figures 109

[11] In respect to the intermingling of the two parental groups the flies thus agree with other insects such as Hemiptera, Orthoptera, Coleoptera, in which an intermingling occurs at least before the adult stage is reached, and presumably much earlier.

[12] These are only of sporadic occurrence, and in my material have been found only in a few cells or in small bits of tissue, never throughout the body.

and 110 represent such prophases, taken from a small bit of somatic tissue, apparently ectodermal, in which practically all of the nuclei contain a multiple group. These nuclei are very easily identified by their size as well as by their chromosome number. No complete metaphase figures were found among these particular cells, so I could not determine whether the nuclei contained quadruple (48) or double (24) the normal number of chromosomes. But the essential point is clear, that in each prophase nucleus the chromosomes appear in only six different aggregates, just as they do in ordinary prophases.[13] This means that here each aggregate is composed of four or eight chromosomes instead of the usual two. In the tetraploid groups two of the four chromosomes are sister halves of the other two, and hence are respectively similar to them in make-up. But all four of these chromosomes associate in essentially the same manner, i.e., paired chromosomes are indistinguishable from sister chromosomes in their manner of association. It is a natural conclusion, therefore, that the paired chromosomes bear much the same qualtitative relation to one another as do sister chromosomes (that they are qualitatively similar) and that their association is dependent upon, although not necessarily caused by, this relation.[14] Such a conclusion is in harmony with the known facts of cytology and genetics which indicate that corresponding maternal and paternal chromosomes are similar in composition.

If the paired association in diploid cells is an expression of the same underlying forces which bring about the association (synapsis) during maturation, the views here set forth are sup-

[13] Only five aggregates are conspicuous because one of the six is composed of the very small chromosomes. Strasburger ('07) obtained tetraploid groups in chloralized root tips of Pisum, and Stomps ('11) found such groups occasionally in Spinacia, but both authors describe the chromosomes as arranged in pairs instead of tetrads. Evidence is lacking on the crucial (prophase) stages, however, and such metaphase figures as are given may readily be interpreted as indicating association in tetrads, even though the association is not close. The question should remain open until tetraploid prophases are studied in these plants.

[14] See Lundegardh '15, who has come to very similar conclusions respecting the bivalent chromosomes of the heterotypic maturation division in plants.

ported by much evidence other than that from the Diptera. Two cases bear such a resemblance to those described in the Diptera that they will be briefly noted here. The most striking is that described by Wilson ('10) in Metapodius, certain individuals, of which possess a supernumerary Y-chromosome or a supernumerary 'm-chromosome'. The extra chromosome in these specimens always "behaves according to its own kind" (p. 69), exhibiting a definite relation to those of its own kind, but to no others, in the pre-reduction stage of maturation.[15] Similarly Miss Woolsey ('15) has found that in a certain specimen of Jamaicana subguttata two small chromosomes act as the synaptic mate of one large bipartite chromosome which has apparently arisen by the union of two chromosomes corresponding respectively to the two with which it associates. These cases, like those of pairing in the Diptera, are readily explained upon the assumption that the association depends upon a qualitative likeness between corresponding chromosomes, but are difficult to interpret otherwise.

At first sight the conclusion that only qualitatively similar chromosomes associate in pairs might seem to be contradicted by the pairing of the unequal XY chromosomes in the males; but the contradiction, I believe, is apparent only. The studies of Stevens on Coleoptera and Diptera, and of Wilson, Payne and others on Hemiptera indicate that the XY pair when present has arisen either from an XY, XY pair, one member of which has lost its X-chromatin, or from a Y, Y pair, to one member of which X-chromatin has become attached.[16] In either case the X-chromosome of the Diptera may be looked upon as a Y-chromosome with X-chromatin added to it; and upon the view that similarly constituted chromosomes associate together the Y-portion of X would be expected to associate with the true

[15] See footnote 20, page 264.

[16] ". . . . we may, accordingly, think of the XY-pair as being essentially a YY-pair with one member of which the X-chromatin is associated." (Wilson '11, p. 87.). Genetic work on Drosophila indicates that the Y-chromosome in this fly is inactive (i. e. no factors have been found in it), but this does not necessarily mean that it is physico-chemically different from the Y-part of X.

Y-chromosome, or in other words the two sex-chromosomes would be expected to associate in approximately the same manner as do corresponding autosomes. In reality the association of the sex-chromosomes differs slightly from that of the autosomes, in that the former appear to condense earlier in prophase and become separated more frequently in metaphase than do the latter, but in essential features pairing is the same in both.

As suggested above the phenomena of chromosome pairing in somatic and primordial germ-cells appear to be closely correlated with those of maturation in spermatocytes and oocytes. The latter phenomena are obviously much more complicated than the former, and the association of the chromatic elements during synapsis is perhaps much more intimate than during the resting stage or prophase of somatic cells; but the similarity between the figures in the somatic cells of flies and those in germ-cells of many animals (including flies) makes it seem very probable that essentially the same cause is operative in both cases. If this be true it would seem that in the development of a fly each cell division is preceded by an attempt at synapsis. Or, in other words, the tendency to undergo synapsis is so marked as to bring about a close approximation of homologous chromosomes during each cell generation.

No positive answer can be given to the question as to why pairing outside the sphere of maturation should be exhibited by some organisms and not by others. Among animals a definite pairing of all the chromosomes in somatic as well as germ-cells is known to occur only in the Diptera. Among plants it has been reported in several orders. But whether the phenomena are really the same in the two kingdoms is not clear, for the details of the process in plants are still obscure. In the Diptera one of the most characteristic features of pairing is the close apposition of the early prophase threads, upon which subsequent behavior seems to depend. Whether a similar apposition is found in the prophases of plant cells, or whether a pairing takes place just preceding metaphase, is not certain. The observations of Stomps on Spinacia, of Overton on Thalictrum

and of Ishikawa on Dahlia strongly indicate that the former is the case in some instances. The first author describes a definite and intimate paired association as a normal condition in vegetative prophases of Spinacia (Stomps '11, p. 258).[17] On the other hand Müller ('12), who gives perhaps the most complete description of prophase stages in any plant exhibiting paired chromosomes (Najas marina), apparently considers the chromosomes to be single (though split) in prophase, and believes the real pairing to occur in metaphase. I am inclined to be skeptical about this interpretation, however, for the dual elements in his prophase figures bear a striking resemblance to those in the Diptera. Unfortunately his prophase figures do not include all of the chromosomes in a nucleus, and it is impossible to tell whether the number of double threads is haploid or diploid.

With respect to the other cases (among plants) in which pairing has been described, the evidence regarding prophase processes is still less satisfactory, and no conclusion of any weight can be drawn from it. The meagre data available from botanical sources tell little about the details of pairing, but they do indicate that it varies in extent or degree among different groups of plants.

In respect to animals a similar generalization may be made, for, although a conspicuous and uniform pairing seems to occur only in the Diptera, yet a varying degree of pairing is discoverable in other organisms. For instance in Hemiptera the 'm-chromosomes' and other morphologically distinct types are frequently associated in pairs, and in the Orthoptera a tendency toward pairing has been noted by Montgomery, Sutton and others.

[17] "Spinacia oleracea hat in den vegetativen Kernen ihrer diploiden Generation 12 Chromosomen aufzuweisen. Diese sind in Paaren angeordnet, und zwar nicht nur innerhalb der Kernplatten (fig. A.) sondern auch, wenn die Chromosomen in den Prophasen an der Kernwand liegen und sehr wahrscheinlich auch im Ruhezustande der Kerne. Denn sobald die Chromosomen sich in der Prophase einer Teilung aus dem Netzwerk des ruhenden Kerns herausgesondert haben (Prochromosomen sicht man im Ruhekern nicht) zeigen sie die paarweise Anordnung und bisweilen kann man beobachten, wenn in irgendeinem Paare ein Teil der beiden Chromosomen noch mehr oder wenier netzförmig ist, dass diese beiden netzförmigen Partien einander deutlich parallel liegen."

Whatever may be the fundamental cause of this phenomenon it seems certain from the evidence that its manifestations differ markedly in different organisms. There seem to be various intermediate conditions between that of intimate pairing (Diptera) and that of very slight pairing. It may be true therefore, that the tendency to associate in pairs is inherent in the chromosomes of multicellular organisms, being manifest in all the cells of some, but only in the maturing germ-cells of others. At all events it seems certain that the Diptera are not sharply differentiated from other animals by reason of any primary distinction in organization responsible for the pairing of their chromosomes.

Many of the questions suggested by this study are intimately involved with those of maturation, and can only be satisfactorily treated when the phenomena of maturation in the Diptera are better known. For this reason a full discussion of them will be reserved for a subsequent paper in which I hope to consider the maturation processes in detail, but in conclusion a word may be said as to the bearing of the pairing phenomena upon the theoretical question of synapsis. It is a significant fact that in the diploid cells of the flies a process may actually be followed which agrees in all essential respects with parasynapsis. In metaphase corresponding chromosomes, although arranged in pairs, are usually not closely applied; but in anaphase the members of these pairs often become associated side by side as they pass toward the poles, until the approximation becomes very intimate.[18] In these cases there can be no doubt about the reality of a process which, whether or not it actually corresponds to that of synapsis, certainly involves the essential features of a synaptic (parasynaptic) union, and removes the a priori objections urged against the conception of synapsis.

[18] With regard to the synapsis during maturation it may be noted that during the final spermatogonial anaphase (and probably oogonial also) the chromosomes behave in this same manner, and hence are brought into a closely paired arrangement before maturation.

SUMMARY AND CONCLUSIONS

1. The chromosomes of about eighty species of Diptera have been examined with especial reference to the phenomena of chromosome pairing. The species studied range from among the lowest to among the highest families in the order. In a large proportion of cases the studies include somatic, spermatogonial and spermatocyte, or somatic and ovarian cells.

2. In all of these species the chromosomes were found to be uniformly associated in pairs in diploid cells. The only irregularities were occasionally displacements involving one or two pairs.

3. The paired association was found to be characteristic of all tissues, somatic as well as germinal.

4. It was found to continue throughout all stages of cell division from earliest prophase to latest anaphase, being most intimate in the earliest and latest stages, and least intimate in metaphase. Telophases and resting nuclei were not favorable for study.

5. Association of paternal with maternal chromosomes apparently is effected in early cleavage stages (perhaps before the first cleavage), since in late cleavage stages the chromosomes are definitely paired.

6. The paired association was found to continue during all stages in ontogeny, from the egg to the adult.

7. Certain cases of multiple chromosome numbers (tetraploid or higher multiples) were found in occasional cells. In these cases corresponding chromosomes were associated together in prophase in aggregates of four, eight, etc., instead of being arranged in pairs.

8. In many species several (in some cases nearly all) pairs of chromosomes could be individually distinguished by characteristics of size and form.[18a] These pairs, with the exception of the sex-chromosomes in males, were in all cases symmetrical, i.e., composed of similar members.

[18a] Since this paper was sent to press I have found a species of Drosophila in which each pair of chromosomes is very clearly differentiated from all others.

9. In certain respects the pairing phenomena were found to present a striking similarity to synaptic phenomena. They give an actual demonstration of a side by side approximation of corresponding chromosomes.

These facts lend strong support to the conclusions:

1. That the paired arrangement of chromosomes is not due to a random assorting process, but is selective to the highest degree.

2. That each maternal chromosome becomes associated with a definite, similar paternal chromosome and with no other.

3. That chromosome pairing is dependent upon the qualitative nature of the chromosomes,—and more specifically upon a qualitative (physico-chemical) similarity between associating members.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures were drawn with the aid of a camera lucida, using a Zeiss 1.5 mm. apochromatic objective and compensating ocular no. 12, with tube length of 160 mm. The drawings are reproduced natural size. They are taken from sections cut 5 μ thick unless otherwise noted.

1 and 2 Drosophila virilis Sturtevant mss.,[19] diploid metaphase, ovarian cell.
3 D. ramsdeni Stt. mss., diploid metaphase, ovarian cell.
4 Scaptomyza graminum Fall., diploid metaphase, spermatogonium.
5 Same, ovarian cell.
6 Same, haploid, second spermatocyte.
7 Drosophila robusta Stt. mss., diploid, early anaphase, spermatogonium; polar view showing separation of daughter halves of chromosomes.
8 Same, ovarian cell; slightly earlier stage showing division of chromosomes.
9 Same; slightly later stage.
10 D. nebulosa Stt. mss., haploid, second spermatocyte prophase.
11 Same, diploid, ovarian cell.
12 Same, diploid, ovarian cell, two poles of anaphase; lower figures displaced in order to compare the two groups (upper pole at left, lower at right); upper figure a diagram showing the two anaphase groups as they appear in the section. The small, spherical members are not evident.
13 D. amoena Loew, haploid, late metaphase, second spermatocyte.
14 Same, ovarian cell, prophase, diploid group.
15 Same, diploid, metaphase, spermatogonium.
16 Same, diploid, early anaphase, ovarian cell, showing separation of daughter halves of chromosomes.
17 D. busckii Coq., diploid metaphase, ovarian cell.
18 Same, haploid, first spermatocyte.
19 D. ampelophila Loew, diploid metaphase, ovarian cell.
20 D. dimidiata Loew, diploid, metaphase, ovarian cell.
21 D. ornatipennis Will., diploid, ovarian cell, metaphase; this individual apparently possesses three small, spherical chromosomes.[20]
22 Scaptomyza adusta Loew, diploid, metaphase, ovarian cell.

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[19] See footnote 3, page 221.

[20] Apparently this case is comparable with that of the supernumerary 'm-chromosome' described by Wilson ('10) in Metapodius, and results from non-disjunction of the small chromosomes in one of the parents. Unfortunately only two or three good figures were found in my specimen (as is usually the case in flies), and although these show the same features they are too few to be demonstrative. It may be noted that the three chromosomes are associated together in each of the figures.

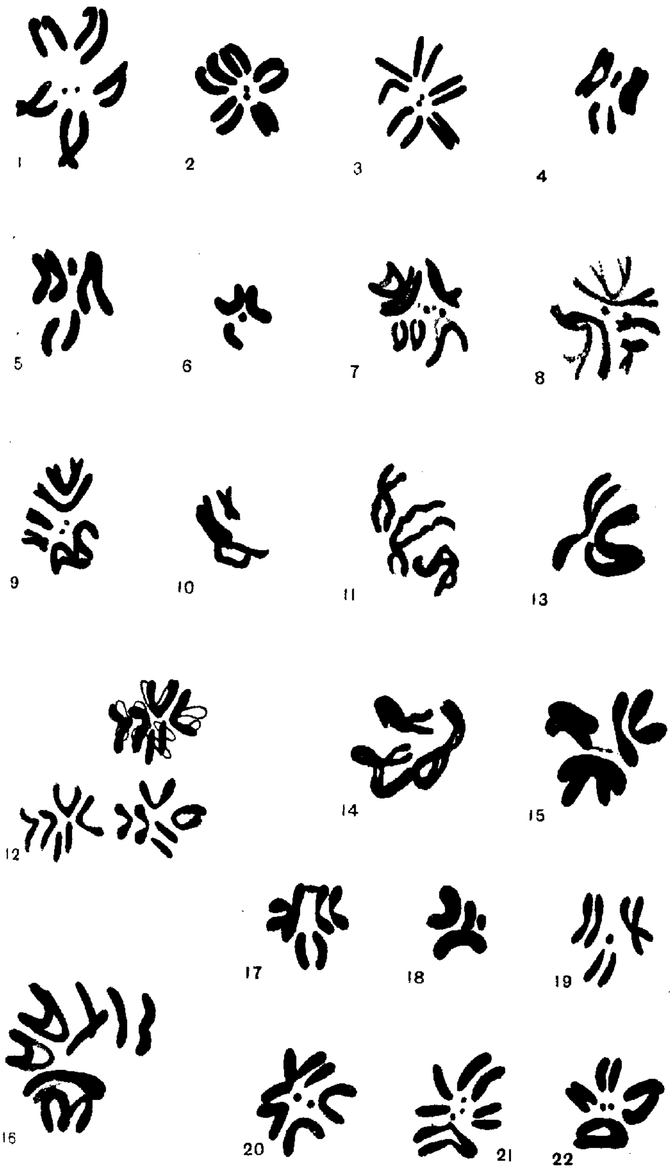
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PLATE 2

EXPLANATION OF FIGURES

23 Drosophila neglecta Stt. mss., diploid, metaphase, spermatogonium; (small chromosomes not evident, unless represented by the small chromatic body in upper part of figure.)

24 Same, all chromosomes present; made from an aceto-carmine smear.

25 and 26 Same, haploid, second spermatocytes; aceto-carmine.

27 D. funebris Fabr., diploid, metaphase, ovarian cell.

28 Same; aceto-carmine smear, slightly later stage showing division.

29 Same diploid, metaphase, spermatogonium; note the separation of the two large chromosomes.

30 Same, diploid, ovarian cell, anaphase in side view; note separation of large chromosomes.

31 Same, with large chromosomes in their normal position.

32 Same, early anaphase, side view, showing division of the chromosomes.

33 Same, haploid, first spermatocyte anaphase in side view for comparison with figure 31.

34 D. procnemis Will., diploid, ovarian cell, prophase.

35 and 36 Same, slightly later stages.

37 D. tripunctata Loew, diploid, metaphase, ovarian cell.

38 Same, diploid, ovarian cell, two poles of anaphase in polar view, displaced for comparison of the two groups.

39 D. repleta Woll., diploid, metaphase, ovarian cell; aceto-carmine smear.

40 Same, late metaphase showing division of chromosomes, from section. The two round bodies at left of figure are chromatic (?) inclusions, not chromosomes.

41 Same, spermatogonium.

42 D. affinis Stt. mss. diploid, metaphase, spermatogonium.

43 Same, late prophase, ovarian cell.

44 and 45 D. obscura Fall. diploid, metaphase, spermatogonia.

46 Same, ovarian cell, (small chromosomes not evident).

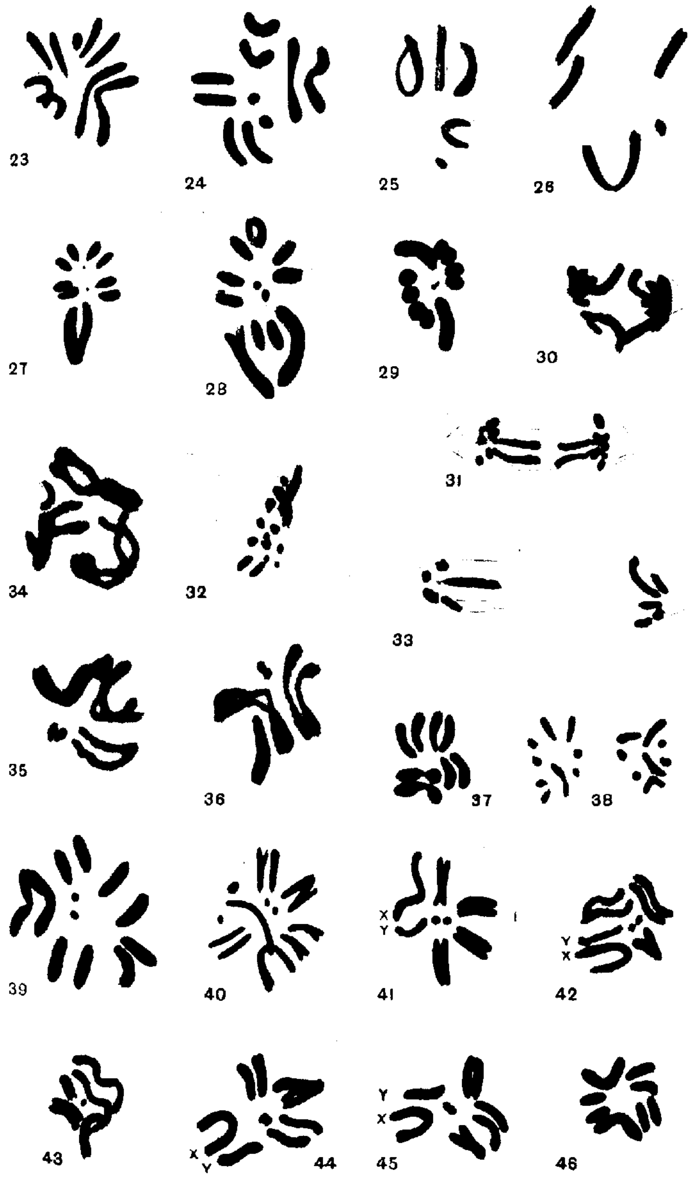
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PLATE 3

EXPLANATION OF FIGURES

47 Drosophila obscura, diploid, late prophase, from an embryonic cell during a late cleavage stage in the egg.

48 Same, haploid, metaphase, first spermatocyte.

49 Same, haploid metaphase, second spermatocyte, X-containing class.

50 Same, haploid metaphase, Y-containing class.

51 and 52 Calliphora erythrocephala, haploid, metaphase, first spermatocytes.

53 to 56 Same, diploid, metaphases, ovarian cells.

57 Same, somatic.

58 Same, diploid, somatic, early prophase, entire, or almost entire nucleus, one pair partly displaced in the figure to show all of the threads.

59 Same, somatic, only part of figure shown.

60 and 61 Same, somatic, two sections of one nucleus.

62 Same, somatic, entire or nearly entire nucleus.

63 Same, ovarian cell, entire nucleus.

64 Same, somatic, only three pairs represented.

65 Same, somatic, entire nucleus.

66 Same, later prophase, ovarian cell, showing separation of the two members of a pair in late prophase.

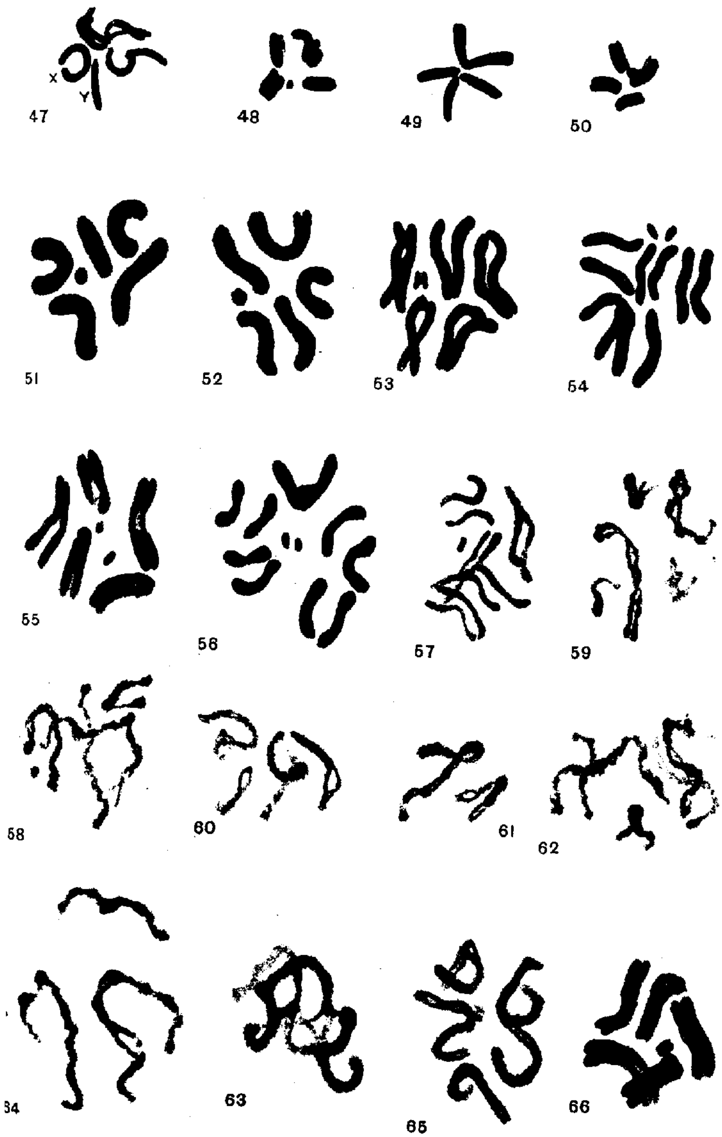
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PLATE 4

EXPLANATION OF FIGURES

68 Musca domestica, diploid, metaphase, ovarian cell.

69 Same, somewhat disarranged.

70 Same, ovarian cell, early prophase, entire or almost entire nucleus.

71 Same, nucleus not entire.

72 Same, late prophase, nucleus almost entire.

73 Phormia regina, haploid, metaphase, X-containing second spermatocyte.

74 Same, Y-containing group.

75 Same as 73, but slightly later, showing division of chromosomes.

76 Same, first spermatocyte, early anaphase, polar view, showing reduction division; note separation of X and Y (small pair).

77 Same, diploid, spermatogonium, late metaphase showing division of chromosomes.

78 Same, diploid, ovarian cell, early prophase.

79 and 80 Same, slightly later stage, nuclei entire, or nearly so.

81 and 82 Sarcophaga tuberosa serraceniae, haploid, second spermatocyte,

83 Same, first spermatocyte.

84 Same, side view, showing separation of X and Y at the reduction division.

85 to 88 Same, diploid, spermatogonia; two small members are X and Y.

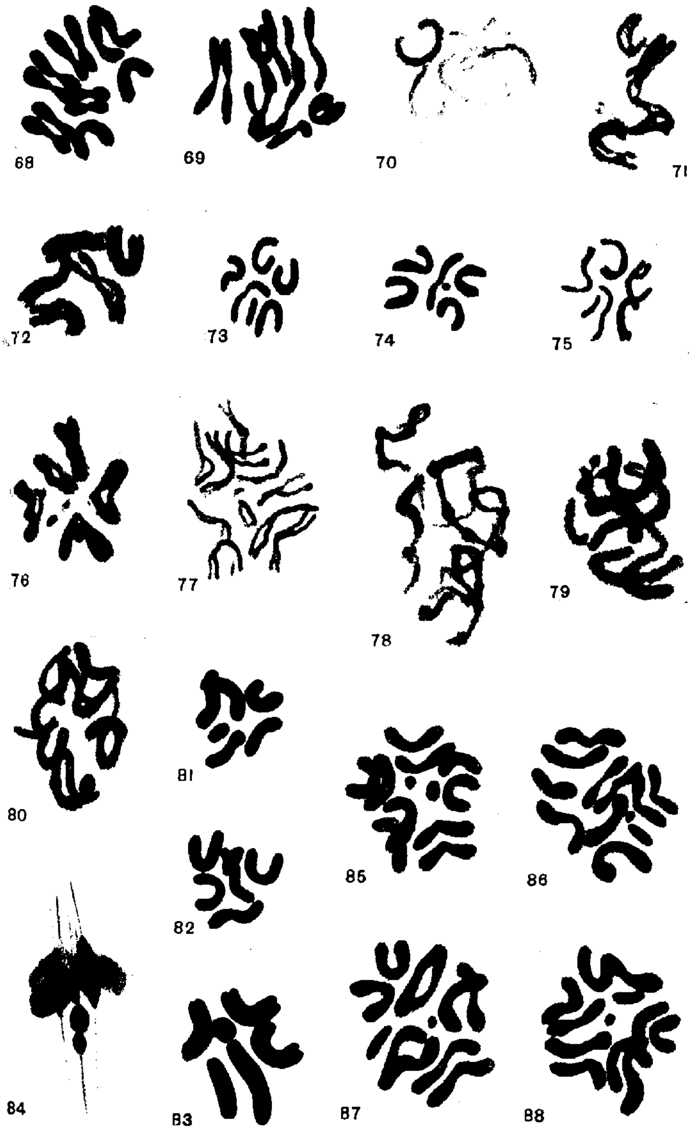
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PLATE 5

EXPLANATION OF FIGURES

89 Sarcophaga sp., diploid, late prophase, ovarian follicle cell.

90 Same, somatic, small pair is XX pair in these figures.

91 Same, somatic, earlier prophase, two sections of one nucleus.

92 and 93 Same, slightly later prophase, entire nuclei.

94 Same, later stage.

95 Same, somatic, anaphase, side view showing both groups of daughter chromosomes; note the closely paired association.

96 Same, tetraploid metaphase; four small chromosomes are X chromosomes, from ovarian follicle cell.

97 Same, multiple group, somatic, apparently containing 48 chromosomes.

98 Ravinia peniculata, diploid, metaphase, ovarian cell.

99 Same, slightly later, ovarian follicle cell, showing splitting of chromosomes.

100 Homalomya sp., diploid, somatic, very early prophase showing bivalent threads, nucleus practically entire; note the polarization.

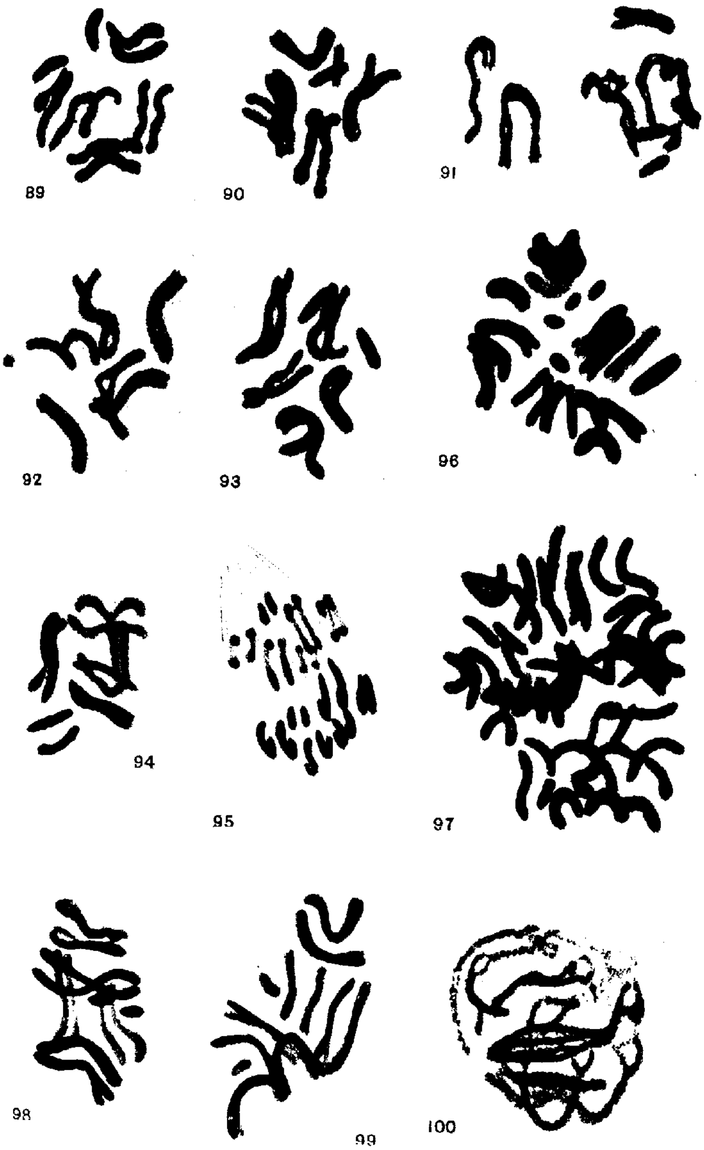
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PLATE 6

EXPLANATION OF FIGURES

101 Homalomya sp., diploid, somatic, early prophase, similar to figure 100; only portion of figure shown.

102 Same, slightly later stage, somatic, larval, nucleus not entire.

103 Same, haploid, prophase; only part of figure shown, second spermatocyte.

104 Same, haploid, second spermatocyte metaphase (late) showing division of the chromosomes.

105 Same, second spermatocyte, anaphase; entire nucleus.

106 Fucellia marina, diploid, somatic, early prophase, entire nucleus.

107 and 108 Same, somatic, later stages.

109 and 110 Same, somatic, prophases, multiple chromosome number, probably tetraploid.

111 and 112 Ophyra leucostoma, diploid, telophases, ovarian cells.

113 Same, spermatogonium, metaphase, showing division of chromosomes.

114 Same, haploid, first spermatocyte (reduction division), compare with figure 113.

115 Neuroctena analis, haploid, metaphase, second spermatocyte.

116 Same, diploid, spermatogonium.

117 and 118 Chaetopsis fulvifrons, diploid, metaphase, spermatogonia.

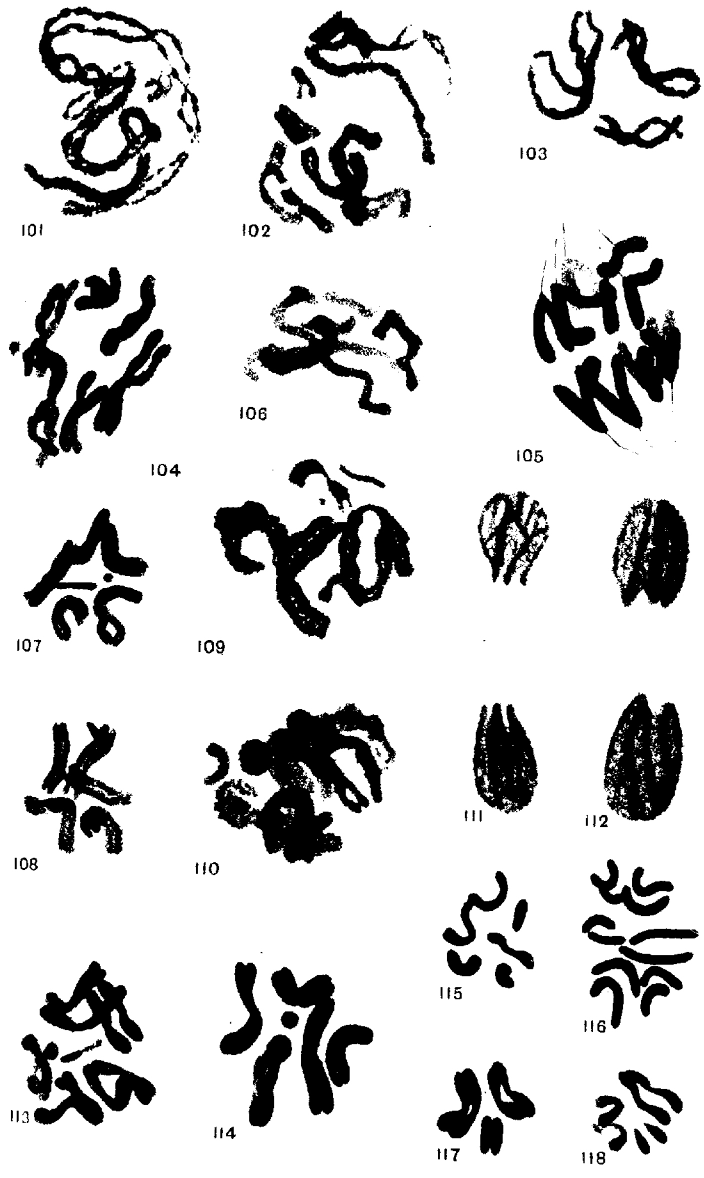
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PLATE 7

EXPLANATION OF FIGURES

119 Chaetopsis fulvifrons, haploid, metaphase,, first spermatocyte.

120 Camptoneura picta, diploid, metaphase, spermatogonium.

121 Same, haploid, metaphase, first spermatocyte.

122 Physegenua vittata, diploid, metaphase, spermatogonium.

123 Eristalis bastardi, diploid, early prophase, spermatogonium; nucleus not entire.

124 Volucella obesa, diploid, metaphase, spermatogonium, one chromosome missing.

125 Same, entire.

126 Same, haploid, first spermatocyte; small bivalent is XY pair.

127 and 128 Mesogramma marginata, diploid, metaphase, spermatogonia.

129 and 130 Anthrax lateralis, diploid, late prophase, spermatogonia; small pair not evident.

131 Same, earlier prophase, nucleus entire.

132 and 133 Same, metaphase.

134 to 137 A. sinuosa, diploid, metaphases, spermatogonia.

138 Same, haploid, first spermatocyte.

139 Same, haploid, second spermatocyte, Y-containing group.

140 Same, X-containing group.

141 Spogostylum simson, diploid, metaphase, ovarian follicle cell.

142 Same, early prophase.

143 Asilus sericeus, diploid, metaphase, spermatogonium.

144 and 145 Same, slightly later metaphases.

146 and 147 Asilus lecythus, diploid, metaphases, spermatogonia.

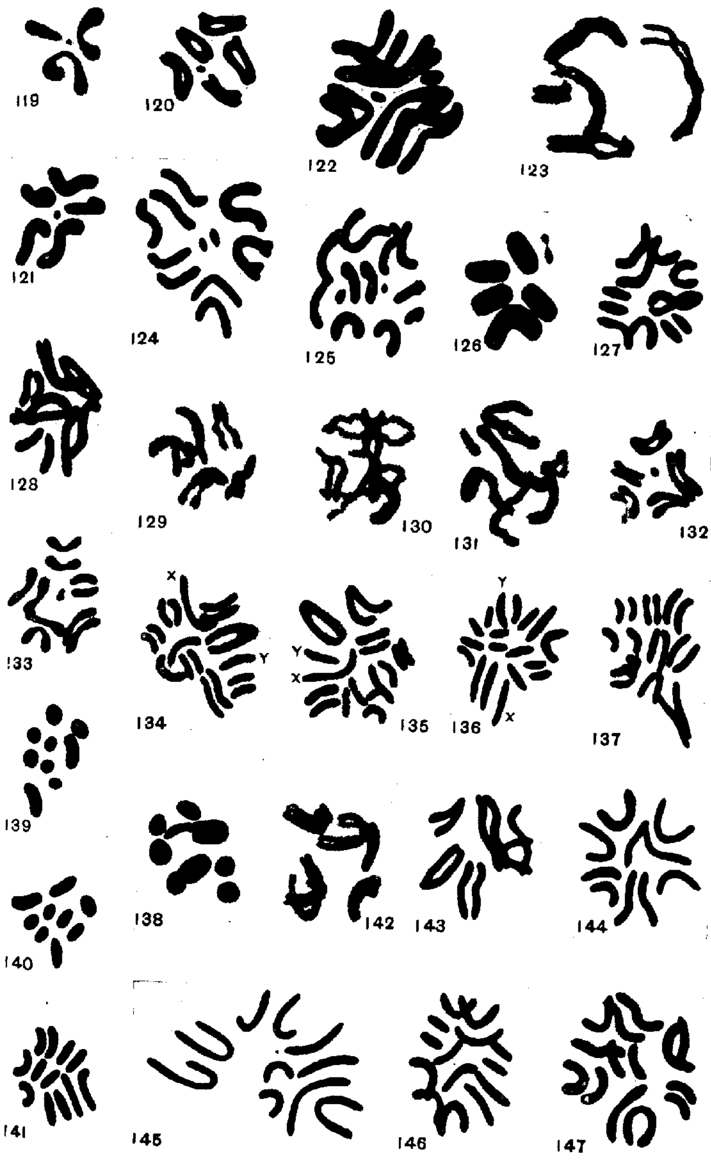
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PLATE 8

EXPLANATION OF FIGURES

148 Asilus lecythus, haploid, metaphase, second spermatocyte.

149 Asilus notatus, diploid, metaphase, spermatogonium.

150 Same haploid, metaphase, second spermatocyte.

151 Leptogaster badius, diploid, spermatogonium, metaphase.

152 Same, haploid, metaphase, second spermatocyte.

153 Erax rufibarbis, diploid, metaphase, spermatogonium.

154 Same, haploid, second spermatocyte.

155 Dasyllis thoracica, diploid, spermatogonium, prophase, entire nucleus.

156 Same, metaphase.

157 Same, haploid, metaphase, first spermatocyte.

158 Same, haploid, second spermatocyte.

159, 160 and 161 Deromyia winthemi, diploid, metaphase, spermatogonia.

162 Same, haploid, metaphase, first spermatocyte; note X and Y going to opposite poles.

163 Same, haploid, second spermatocyte, Y-containing group.

164 Same, X-containing group.

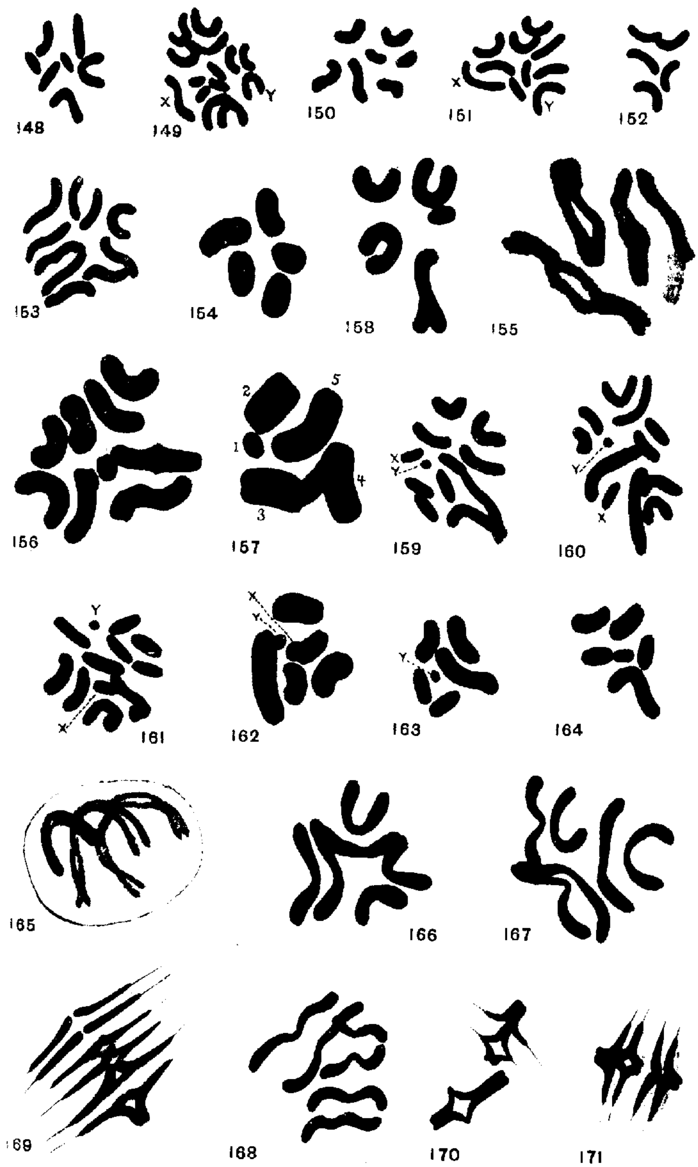
165 Culex pipiens, diploid, early prophase, ovarian cell, entire nucleus. Three pairs of chromosomes, note polarization.

166, 167 and 168 Same, diploid, spermatogonia, metaphase.

169 Same, anaphase, side view showing division of chromosomes and separation of daughter halves.

170 Same, earlier stage, side view showing manner of division; only three of the chromosomes are represented.

171 Same, same stage as 169; only two pairs of chromosomes represented.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OBSERVATIONS ON CILIARY CURRENT IN FREE-SWIMMING PARAMECIA

S. O. MAST AND K. S. LASHLEY

From the Zoölogical Laboratory of the Johns Hopkins University

SIX FIGURES

INTRODUCTION

When paramecia are at rest and are feeding, a cone-shaped current from a considerable distance in front of the animals proceeds down the oral groove toward the mouth. This current may be called a feeding-cone or merely a cone. Similar currents are sometimes seen when the animals approach a region containing particles of carmine or India ink. When this occurs the particles in suspension are drawn toward the paramecia from in front in such a way that they appear to come in contact with the oral groove before the anterior end reaches the original boundary of the region. Jennings demonstrated this very clearly ('06, p. 45). He maintains that this phenomenon occurs not only when the paramecia approach a foreign substance but that it occurs continuously in free-swimming animals, and that it serves them in detecting in advance the condition of the environment they are about to enter, thus making it possible to avoid injurious substances and unwholesome regions, without actually getting into them. He says ('06, p. 47),

Paramecium is continually receiving 'samples' of the water in front of it. Since in its spiral course the organism is successively pointed in many different directions, the samples of water it receives likewise come successively from many directions (fig. 33).[1] Thus the animal is

[1] This figure represents a paramecium proceeding on a spiral course, and continuously drawing in from some distance in front, a cone-shaped current. It has been extensively copied, especially in general zoölogical works. If our conclusions are correct the shaded regions representing currents in this figure should be omitted.

given opportunity to 'try' the various different conditions supplied by the neighboring environment. Paramecium does not passively wait for the environment to act upon it, as Amoeba may be said, in comparison, to do. On the contrary, it actively intervenes, determining for itself what portion of the environment shall act upon it, and in what part of its body it shall be primarily affected by the varying conditions of the surrounding water. By thus receiving samples of the environment for a certain distance in advance, it is enabled to react with reference to any new condition which it is approaching, before it has actually entered these conditions.

Several years ago, in making observations under the binocular on paramecia in rather large quantities of solution containing numerous particles in suspension, the senior author was surprised to find no evidence whatever of a movement of particles toward individuals which were swimming freely. As seen under the binocular with a magnification of 40 to 60 diameters, there is, under such conditions, no appreciable movement of the particles either in front of or along the sides of the paramecia. If pronounced water currents were drawn in continuously from in front of the animals, as is maintained by Jennings, one would expect, with numerous paramecia swimming about in every direction, to find the liquid about them churned into violent motion, but this is not the case; quite the contrary; the liquid under such conditions appears to be stationary. The paramecia, gliding about among the particles in suspension disturb them surprisingly little.

These contentions are supported by the results of more extensive observations by the junior author. Most of these observations were made on Paramecium but some were also made on other organisms.

OBSERVATIONS ON PARAMECIUM

The ciliary currents produced by free-swimming paramecia were studied first in a watch glass containing approximately 1 cc. of fluid, consisting usually of a dilute suspension of India ink in fresh culture fluid to which the animals had become acclimated. Under these conditions most careful observations both under a binocular and under a compound microscope failed to

reveal in specimens swimming freely any evidence of the production of feeding cones. Under certain conditions such cones are, however, produced in free-swimming animals as will be shown later.

The currents produced by free-swimming paramecia were studied by fixing the attention upon single particles just in front of them and watching the movements of these particles until the animals had passed. The absolute movement of the various particles is extremely difficult to ascertain owing to the rapid movements of the animal, but certain points come out very clearly. Particles in front of the animals are not set in motion until they are within less than twice the length of the animal's anterior cilia. They are then drawn toward the advancing animal until they are apparently in contact with its cilia when they are thrown rapidly to one side and are carried back along the surface of the animals, those in the oral groove moving considerably more rapidly than the rest. The current in the oral groove, however, although stronger, does not draw particles from in front to any greater extent than does that of the aboral side. The more rapid movement of water in the oral groove is compensated by lateral movements of the anterior end of the animal resulting in the spiral course, and there is no indication whatever of the formation of a cone. The direction and relative rate of movement of the particles surrounding the paramecia compared with the rate of movement of the paramecia are represented in figure 1.

In ordinary cover-glass preparations under the monocular microscope the paramecia very frequently appear to draw in a constant cone-shaped stream of water from the front just as has been described for animals which are at rest and are producing feeding-cones. This may be observed either by placing them in a dilute suspension of India ink or carmine or by adding ink at one side so that it forms a dense, sharp-edged cloud into which the animals swim. Under these conditions, however, the cone is not always produced—many animals approach and enter the cloud of ink without seeming to displace the particles of ink in the least until the cilia on their anterior ends come into

actual contact with the particles. But in regard to the manner of swimming no difference could be detected between those which produce the cone and those which do not.

One of the chief causes of the occasional production of the cone under the above conditions, can readily be detected in observations with the binocular. The ordinary cover-glass mount includes a layer of water which is scarcely thicker than the width of the spiral course taken by the paramecia. The animals consequently strike against the slide or cover-glass at almost every

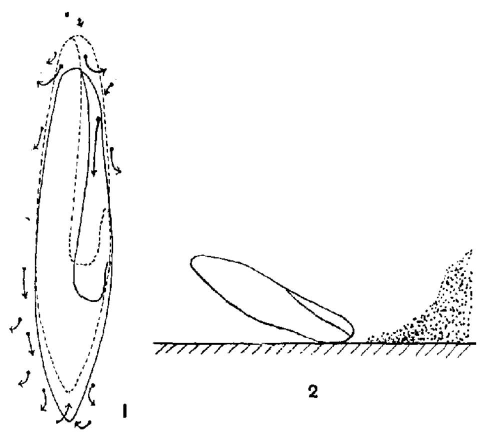


Fig. 1 Diagram illustrating the movement of particles produced by Paramecium swimming freely in a dilute suspension of India ink: a composite of many observations in which single particles were selected and kept in view as the organism swam past them. The dots represent the positions of particles when the paramecium had the position represented by the continuous outline: the arrows represent the direction and the extent of movement of the particles during the time required by the paramecium to move from the original position to that represented by the broken outline. Note that the particles in front of the paramecia do not move until the anterior end comes very near them.

Fig. 2 The production of a feeding-cone due to retardation caused by contact with the substratum. The ink spreads most rapidly along the substratum and, when the edge of the cloud is brought into sharp focus, only those animals are visible which are swimming near the bottom. These frequently come in contact with the substratum and the cone results from the consequent mechanical retardation of the animal.

revolution of the spiral. The action of the cilia against a solid seems to be less effective than the free beat in the culture fluid and so the animals are retarded at each contact. If this takes place just before the animal enters the edge of a cloud of ink the continued beating of the strong cilia of the oral groove, no longer compensated for by the forward or lateral movement of the body, draws a funnel shaped mass of water from in front, which may reach or exceed the length of the body. The pause in the animal's forward progress is only momentary and is quite easily overlooked, but it can be distinctly seen. Even in deeper preparations, in watch-glasses, where the organisms may swim freely without contact with the bottom of the dish the conditions of observation are frequently such as to give the impression that the cone is produced much more frequently than is in reality the case. When a dilute suspension of India ink is used to form a sharp edged cloud in the culture dish, the ink rapidly settles and spreads out along the bottom of the dish so that the edge of the cloud can be seen only with a deep focus of the microscope. Hence only those animals which are swimming near the bottom are visible and their frequent contact with the bottom subjects them to almost constant mechanical retardation (fig. 2). But retardation owing to contact with the substratum is not the only cause of the formation of feeding-cones in moving paramecia.

The water currents produced by the cilia of Paramecium have by various investigators been most thoroughly studied in animals whose movements were mechanically retarded so that the movements of the cilia might be observed. This is generally brought about by the addition of gelatin or some other colloid to the water. The writers made extensive observations under such conditions with the following results and conclusions:

Animals in quince-seed jelly so thick that they force their way through it with difficulty, always produce apparently continuously, a very marked feeding cone, whether they are swimming forward or backward; and in dividing animals which lack the oral groove the cone is quite as large as is that produced by normal animals. Such jellies are, however, never homogeneous and

the cone seems to result from the fact that the organisms force their way with difficulty between the masses of denser jelly while the more fluid parts of the preparation, with the granules which they contain, are easily set in motion and sucked towards them. After the animals have been in the jelly for a short time it is cut up into canals, each marking a place through which an animal has previously passed. These are indistinctly visible and may be traced for some distance through the preparation. The animals tend to follow these open paths where they meet with less resistance and in swimming through the less dense medium they produce practically no cone. In giving the avoiding reaction under these conditions they draw in a cone of water from behind momentarily but if the backward swimming follows the path along which they have just advanced the cone disappears as soon as the rate of locomotion becomes uniform; only when they force their way through the denser jelly do they produce the cone.

Dense suspensions of starch grains, carmine and India ink act in the same way as the quince-seed jelly. The ordinary culture fluid from which the animals are usually taken for study often contains transparent masses of bacteria which are visible only with dark ground illumination. These probably also have the same effect as the jelly in retarding the animals and producing the cone; and it is highly probable that the cones observed and recorded in many instances were caused by these invisible but resistant substances, locally distributed throughout the solution, and that the cones were produced only when the animals entered the regions containing them. Thus it is evident that mechanical retardation is one of the principal causes of the formation of feeding-cones in moving paramecia. They are, however, also produced without any such retardation as will be shown presently.

In some cases when the reactions of the paramecia at the edge of a cloud of ink were being observed, the animals were seen to draw out a cone of ink under conditions where they were certainly not mechanically retarded. This happened most frequently when the animals either paused before entering the cloud or turned aside without entering. In the latter case, which

is in reality a weak avoiding reaction, it appeared that the cone was produced after the animals began to turn aside, rather than before. To test this a small amount of hydrochloric acid was added to the ink-suspension so that the animals gave a weak avoiding reaction at the edge of the freshly added ink. In mixed cultures the reactions of different paramecia to such a preparation differ greatly. Some give a violent avoiding reaction, others enter the acid without a pause. Many, however, give a weak avoiding reaction, i.e., they stop and swerve toward the aboral side without swimming backward. These animals always draw out a cone-shaped mass of ink from the edge of the cloud.

The ciliary movements in reactions of this sort were studied in detail and this gave a clue to the method by which the cone is produced. The animals, without producing a cone, swim up to the edge of the region containing acid until the anterior end is in contact with the ink particles; then the body cilia reverse, but those of the oral groove continue to beat backward. In consequence the forward progress is stopped and the animal swerves to the aboral side. Since the body is prevented from advancing (owing to the reversal of its cilia), the backward stroking cilia of the oral groove draw out a cone of ink which follows the swerving animal until forward progress is resumed as represented in figure 3.

To demonstrate this reaction methylene blue is most favorable. Crystals dropped upon the surface of the water containing paramecia sink to the bottom, dissolving slowly and forming dense blue vertical columns of solution. The paramecia respond to this with a weak avoiding reaction, but they do not respond until after the anterior end has actually come in contact with the blue solution, then they turn and the blue solution is drawn out in the form of a cone as previously described. The feeding cone is, however, not always produced when paramecia respond to acids.

After a drop of acidified ink has been in a culture of paramecia a few minutes, they no longer respond to it as they did at first. They collect in a zone around it and swim far into the cloud before giving a reaction; then they respond with a strong avoid-

ing reaction which is not accompanied with the production of a feeding cone. Under these conditions the cone, however, is sometimes produced as the animals enter the cloud, although there is no apparent turning aside as in the typical weak avoiding reaction. The cone in such cases is usually small and the animals frequently seem to pause just before it is produced. The reaction is relatively infrequent and it could not be studied under a magnification great enough to reveal the ciliary movements, but in some cases the pause in the animal's progress just

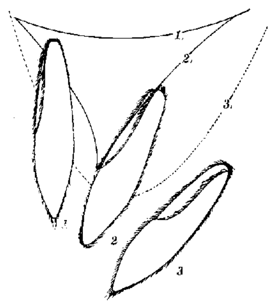


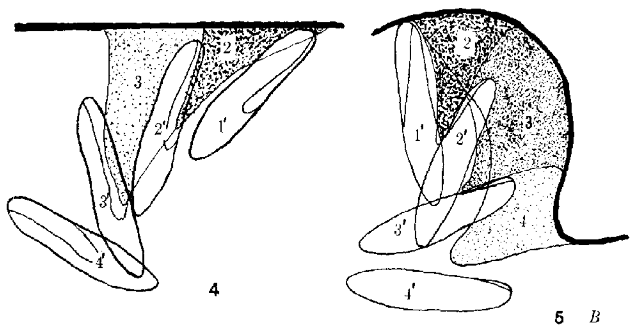
Fig. 3 Illustrating the production of the feeding-cone during the weak avoiding reaction. *1*, *2*, *3*, successive positions of the paramecium; *1'*, *2'*, *3'*, corresponding positions of the edge of the cloud of ink. The paramecium, weakly stimulated by the edge of the cloud of ink, reacts by reversing the cilia of the body, but not those of the oral groove. Currents of water are drawn in from both anterior and posterior directions but most strongly along the oral groove where the currents are visible as a cone in the movements of the edge of the cloud of ink. Note that the feeding-cone is not produced until after the paramecium begins to turn.

before the formation of the cone was unmistakable and indicated that the formation of the cone was due to an avoiding reaction so weak that there was no deflection in the course of the animal.

The absence of feeding cones in strong avoiding reactions is especially striking in responses to alkalies. If, for example, strong potassium hydrate is added to the cloud of ink the animals, without producing a cone, swim forward until the anterior end penetrates the edge of the cloud, then they reverse all their cilia and swim backward violently, leaving a deep depression in

the edge of the cloud. At no time during the reaction or before is the ink drawn out in the form of a cone. This fact strongly supports our contention that the cone is not continuously produced in free-swimming paramecia.

A few observations seem to indicate that in the weak avoiding reaction the animals continue to turn as long as the current produced in the feeding cone carries stimulating substance to the oral groove. Usually the weak avoiding reaction occupies but



Figs. 4 and 5 A paramecium respo[illegible] weak avoiding reaction after coming in contact with acidified ink. *1′, 2′, 3′, 4′*, successive positions of the paramecium; *2, 3, 4*, corresponding positions of the feeding cone. Note that the feeding-cone is not produced until after the anterior end has come [illegible] actual contact with the ink and that the paramecium stops turning as soon [illegible] ink no longer comes in contact with it.

the briefest interval. The paramecia swim up to the edge of a cloud of ink, turn rapidly to one side and swim away, leaving a streamer of ink at the edge of the cloud, all so quickly that the details of the process can not be observed. Occasionally, however, the reaction takes place more slowly and in some such cases it seems that the aboral rotation persists just so long as the current of acidified ink comes in contact with the oral groove, and that the backward stroke of the body cilia is resumed as soon as stimulation of the oral groove ceases.

Two such reactions are represented in figures 4 and 5. Both were observed when the majority of the paramecia in the prepa-

ration were turning through only a small angle such as that shown in figure 3. In the reaction represented in figure 4, the aboral side of the anterior end of the organism first came in contact with the acidified ink, then the animal began to turn toward the aboral side and swim backward. Soon after this the ink was drawn out in the form of a cone which increased in size and followed the animal as it backed away, but only for a short time. As soon as the ink no longer came in contact with the paramecium it stopped backing and turning, the cilia reversed and it swam away. In the reaction represented in figure 5, the organism entered a depression in the edge of the cloud of ink and was stimulated while at right angles to this edge. The aboral swerving continued only until the paramecium was carried out of the depression and until the cone no longer reached the body.

A large number of cases of this sort were observed but no conclusive evidence that the degree of turning resulted from the duration of stimulation was obtained. In the same preparation different paramecia give quite different reactions to the same cloud of acidified ink. Some swim far into the cloud before giving any reaction, some give a weak avoiding reaction at the edge of the cloud, and some give a violent avoiding reaction at the first contact with the ink. Jennings has shown that the second phase of the strong avoiding reaction, the aboral rotation, may be continued for a long time after violent stimulation and it is possible that the weak avoding reactions in which the degree of turning seemed to be controlled by the position of the cone were in reality due merely to the chance intensity of stimulation. The proportion of cases in which forward swimming seemed to follow the removal of the stimulating agent from the oral groove is, however, large enough to make an explanation on the basis of chance rather doubtful.

OBSERVATIONS ON STENTOR, SPIROSTOMUM AND ROTIFERS

Free-swimming stentors do not ordinarily produce a feeding cone unless they are mechanically retarded, as often occurs owing to the collection of débris on the mucus so copiously secreted by these organisms, especially at the posterior end. In approaching

a region containing ink no cone is observed until after the anterior end has actually reached the original boundary of the ink and has come in contact with the particles in it. When this occurs the animal usually responds with a weak avoiding reaction, and in turning a cone is drawn out just as in the case of Paramecium. The cone is produced by a modification in the usual motion of the cilia on the disk, such that those on the aboral side strike forward, while those on the opposite side strike backward. This causes the animal to turn toward the aboral side and at the same

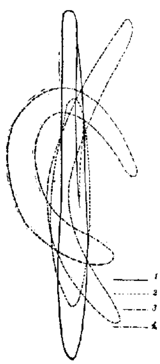


Fig. 6 Diagram illustrating the avoiding reaction in Spirostomum. *1, 2, 3, 4*, successive positions assumed during the reaction.

time produces a cone-shaped current which proceeds from some distance in front of the disk toward its outer oral edge and back along the side of the body.

In free-swimming Spirostomum there is no observable cone if the body is straight, but if it is curved so as to offer considerable resistance there is a decided cone. This, however, was observed only under a cover-glass, where it was impossible to eliminate the probable effect of contact. When the animal comes in contact with a cloud of ink it responds usually with a momentary cessation of ciliary activity and a marked contraction of the body which immediately expands again, taking a somewhat curved form, after which a large feeding cone is pro-

duced. If the cone contains the stimulating agent the organism again contracts after which it very soon expands again, but now it bends much more than it did previously so that the anterior end is usually no longer directed toward the region containing the stimulating agent (fig. 6). The production of the cone in these reactions seems to be due in part to the inertia of the body and in part to the additional resistance due to the curvature in it.

Free-swimming rotifers regularly produce a cone which, however, is rarely more than one-fourth of the length of the body. When they enter an injurious solution they contract the disk and settle to the bottom. Then they usually expand the ciliary lobe but not the entire disk, draw in a small cone of water, turn through a certain angle and contract again. Thus they continue contracting and turning after each stimulation until they are no longer stimulated by the cone, when they expand the disk and proceed.

DISCUSSION

We have thus demonstrated that the feeding-cone in Paramecium, Spirostomum and Stentor is produced only under certain conditions, that during locomotion it is produced only (1) when there is an acceleration in rate without any change in the relative activity of different cilia, (2) when there is a reversal of the cilia, except those in the oral groove and (3) when the animals are mechanically retarded by lateral contact with the substratum, etc. We have also demonstrated that in these organisms the avoiding reaction is not due to the production of a feeding cone. The feeding cone can not, therefore, be of any considerable value in ascertaining the condition of the environment ahead as maintained by Jennings.

These facts do not, however, overthrow the contention that Paramecium continuously tests the condition of the environment ahead. The anterior end of these creatures is undoubtedly sensitive to practically all agents to which they respond, and they usually respond as soon as this end reaches the stimulating agents. Thus they detect favorable and unfavorable regions before they

actually enter them, just as they would if the feeding cone were continuously produced, but not quite so soon. In other words, the reactions in Paramecium, Spirostomum and Stentor are, according to our views, precisely the same in principle, though not quite so efficient as they would be if the feeding cone were continuously produced. However, the contention (Jennings, 1904, p. 449) that the spiral course of these organisms is of particular value in testing the condition of the environment in many directions, is questionable.

SUMMARY

1. Free-swimming Paramecium, Stentor and Spirostomum do not continuously produce a feeding-cone. Water is sucked toward them from in front through only a very short distance, probably a distance not over twice the length of the cilia. This distance is not great enough to make any warning of unfavorable environment ahead, which may be due to such currents, of any appreciable value.

2. The feeding-cone is produced by these organisms: (a) when they are at rest and are feeding; (b) during locomotion if they are retarded by lateral contact with resistant substances which are not uniformly distributed, as e.g. bacterial masses, the substratum, etc., or if the rate of locomotion is increasing or if it is decreasing, provided the decrease in rate is due to a reversal or a decrease in the activity of the cilia on the body without a similar change in the activity of those in the oral groove; (c) in the avoiding reaction, but not until after the stimulus which causes such reactions has been received and the animals begin to turn. It is not the cause of the avoiding reaction.

3. In free-swimming rotifers the feeding-cone appears to be continuously produced.

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THE GROWTH OF THE BODY AND ORGANS OF THE ALBINO RAT AS AFFECTED BY FEEDING VARIOUS DUCTLESS GLANDS (THYROID, THYMUS, HYPOPHYSIS, AND PINEAL).

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FOUR CHARTS

CONTENTS

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I. INTRODUCTION

The present investigation was undertaken in the hope of throwing further light upon the relations of some of the ductless glands to the growth process in albino rats. During the experiment it became evident that the growth rate in the control rats was in many cases somewhat different from that which has been generally described as normal, so it became necessary to include incidentally the question of the normal growth rate.

The investigation was carried on in the Anatomical Institute of the University of Minnesota, under the direction of Professor C. M. Jackson, to whom my grateful thanks are due for his constant interest in the work and his many very helpful suggestions.

Since the ductless or endocrinous glands were first recognized anatomically, various methods have been applied in investigating their functional significance. The four most commonly used, are extirpation of the glands, their transplantation, injection of their extracts and the feeding of the glandular substances.

Feeding ductless glands in order to study the effects of the hyperactivity thus possibly produced has certain objections. The absorption is slower than when injections are made, and the danger of infection is lessened; but the substances fed may undergo digestive changes in the alimentary tract. That the active principles of the ductless glands are not necessarily destroyed by digestion, however, is proven by abundant experimental and clinical results (Gudernatsch '12, and Abderhalden '15 especially in thyroid feeding). The feeding method was selected for the present investigation.

II. LITERATURE

A general discussion of the literature of all of the ductless glands is given by Vincent ('12), "Internal Secretion and the Ductless Glands," and in the more extensive work of Biedl ('13), "Innere Sekretion." The hypophysis literature has been reviewed by Cushing ('12). "The Pituitary Body and its Disorders;" and the work on the thymus has been considered recently by Basch ('13), "Beiträge zur Physiologie und Pathologie der Thymus." A complete list of references to work done upon the albino rat is given by Donaldson '(15).

A preliminary report of the principal results of the present investigation has already been published (Hoskins '16).

Thyroid experiments

Iscovesco ('13) found that daily injections of thyroid extract stimulated growth slightly in young animals but decreased the weight of old animals. He found nearly 100 per cent hypertrophy (measured in grams per kilogram body weight) in various viscera. The extreme and uniform hypertrophy of the organs and especially that of the uterus is difficult to understand. The fact that the liver and the female kidney show no overgrowth is remarkable.

Magnus-Levy ('95) found that feeding thyroid may cause loss in weight in an animal.

Cunningham ('98) fed 'considerable amounts' of thyroid to various animals without noting any toxic effects.

Moussu ('99) reported that small doses of thyroid stimulate the rate of growth in young dogs, but that large doses are toxic.

Rudinger, Falta and Eppinger ('08) and Kostlivy ('10) found that feeding thyroid stimulates the suprarenal glands.

Bircher ('10 a) ('10 b) fed thyroid to young rats and found a retardation in growth and body weight, but an acceleration in the process of ossification.

Utterström ('10) reported an enlargement of the thymus in thyroid-fed rabbits.

Hoskins ('10 a) fed daily, for 15 days varying amounts (5–15 mg.) of thyroid to 18 young guinea pigs. Their suprarenal glands

were 25 per cent heavier than those of 18 controls. The same author ('10 b) fed thyroid to pregnant guinea pigs. Many abortions and several still-born occurred. The newborn (apparently normal) young of the treated mothers weighed on the average 12 grams less than the controls. The hypophysis showed an average decrease in weight of 10 per cent, the suprarenals 26 per cent and 2 per cent for the females and males respectively; the ovaries 26 per cent; and the thyroid gland 18 per cent. The thymus was increased 58 per cent.

Carlson, Rooks and McKie ('12) and Ferrant ('13) fed thyroid to birds and mammals, including man. They concluded that large doses of thyroid are toxic. In the thyroid fed rabbits of Ferrant the heart, liver and pancreas showed degenerative changes.

Schäfer ('12) fed thyroid to young white rats, noting an increased food consumption, increased metabolism and acceleration of growth.

Gudernatsch ('12, '14) found that thyroid administered to a large number of tadpoles retarded growth (i.e., a toxic effect) but hastened metamorphosis of the limbs and tail. It is probable that this acceleration is due to an increase in the rate of the circulation and general metabolism. Lenhart ('15) reaches the same conclusion. The present writer, in a similar (unpublished) experiment (with larval frogs and Ambystomae) fed large doses and noted only the toxic effect. Coutronei ('14), West ('14), Morse ('15) Abderhalden ('15) and Romeis ('15) have recently confirmed in general the results of Gudernatsch.

Hewitt ('14) fed thyroid gland to white rats, noting a loss in weight.

Livingston ('14) found that feeding thyroid inhibits the hypertrophy of the hypophysis which follows thyroidectomy in male rabbits.

Gudernatsch ('15) fed thyroid to albino rats. This treatment retarded growth and interfered with pregnancy. The effect produced is probably due merely to the toxicity of thyroid, as Stockard ('13) obtained quite similar results in animals treated with alcohol.

Thymus feeding

For complete literature see Vincent ('12), Biedl ('13), Basch ('13) and Paton ('13).

Gudernatsch ('12) ('14) found that a thymus diet stimulated body growth in tadpoles, but retarded metamorphosis of the limbs and tail. Similar results in some cases were obtained by Romeis ('15) and Abderhalden ('15).

Gebele ('11) and Miss Hewer ('14) reported negative results with thymus feeding. In the latter's work, thymus, when fed (1 to 4 g. daily) to males, retarded the development of the testes in young rats and caused degeneration of the testes in adults.

Salkind ('15) reported stimulation of growth from feeding large amounts of thymus.

Hypophysis experiments

Cushing ('12) concludes that total loss of the anterior lobe of the hypophysis is followed by death and that partial loss is followed by obesity, sexual infantilism, and retardation of skeletal growth.

Frequent injections of hypophysis extract interfere with growth, (Cerletti '09, Fodera and Pittau '09). A loss in weight with no skeletal changes is reported by Franchini ('10) and by Crowe, Cushing and Homans ('10). A gain in weight after continued injections of hypophysis extract is reported by Delille ('09), and cardiac hypertrophy by Etienne and Parisot ('08); but Caselli ('00) obtained negative results.

Retardation in growth (especially skeletal) as a result of hypophysis feeding was reported by Thompson and Johnston ('05), Etienne and Parisot ('08), Sandri ('07), ('09) (posterior lobe), and Cushing and Goetsch (cited by Cushing ('12), Wulzen ('14) and by Pearl ('16) (anterior lobe) Abderhalden ('15), ana Robertson ('16).

Negative results from feeding hypophysis are reported by Caselli ('00), Sandri ('09) (anterior lobe), Hoskins ('11), Aldrich ('12a) ('12b), Schäfer ('12), Lewis and Miller ('13), and Gudernatsch ('14).

Schäfer ('09) had thought that feeding small amounts of anterior lobe of the hypophysis favors growth. Goetsch ('16) from very few data reports that excessive dosage retards growth in young rats, whereas smaller dosage accelerates growth in body weight and especially the development of the sexual system; that this acceleration is due to the anterior lobe; and that feeding posterior lobe does not accelerate growth, and even retards sexual development. Robertson ('16a) reports that hypophysis extract accelerates growth after inanition.

So far as the literature shows, in none of the above experiments with hypophysis substance was a complete autopsy performed or complete histological examination made. Hallion and Alquier ('08) report hypertrophy of the suprarenals in rabbits, and no changes in the gonads. Wulzen ('14) noted in chickens fed fresh hypophysis that the long bones were shorter than in her control animals, and that the involution of the thymus was hastened by the treatment.

Pineal experiments

In experiments on growth, Dana and Berkeley ('13) reported that after injecting and feeding a few young animals with pineal substance, they noted growth acceleration. These results may be due to the pineal medication or merely to normal variation. Berkeley ('14) reported mental and physical improvement in backward children to whom pineal substance was administered.

Priore ('15) reports that young rabbits into which pineal extract was injected frequently, grew slightly more than the controls, but his results are probably within normal variability. In an older group the controls outgrew the experimental group. In none could any skeletal alterations be seen.

McCord ('14) fed pineal substance to a few chicks, young guinea pigs and pups. He reported an acceleration in their rate of growth, increased mentality in the dogs, and sexual precocity in some of the guinea pigs.

The same writer ('15) later reported that the growth of young guinea pigs which received daily 10 mgms. of pineal substance from adult cattle was delayed. Adult guinea pigs were not affected by calf pineal substance (dried), but the growth rate

of young guinea pigs was accelerated by injections of extract of calf pineals. Pineal substance hastened sexual development in guinea pigs. Males were more susceptible than females to pineal treatment. The treated animals did not grow larger than normal adults and their growth was apparently proportional in all parts of the body. The 'sexual precocity' noted by McCord perhaps can be explained by the fact that in growing animals the sexual development normally tends to keep pace with somatic growth. In stunted animals sexual maturity is retarded as seen in inanition experiments (Jackson '15); conversely it is to be expected that a hastened somatic growth is accompanied by a corresponding development of the reproductive organs. This condition can hardly be called sexual precocity, especially if the sex organs are not relatively overdeveloped.

Dandy ('15) was able to remove the pineal body from young dogs with no apparent after-effects.

Many experimental data that have been published are worth very little to us because of incomplete records and also because animals of one strain kept under certain conditions have been used as control animals and checked against experimental animals of a possibly different strain kept perhaps under different conditions. It is also true that in many experiments, although all the animals are of the same strain and are kept under similar conditions, only a small number are used and these represent different litters. Since it has been shown by Jackson ('13) and King ('15) that in albino rats variability in body weight within a litter is only about half as great as general racial variability, the advantage of taking controls and experimental animals from the same litter is obvious. The apparent 'results' obtained in many experiments may very well be due merely to the above-mentioned factors and not to the experiment itself.

III. MATERIAL AND METHODS

The albino rat (Mus norvegicus albinus) was selected for the experiment because it is a convenient form for use and more is known about the growth of this animal than of any other, owing to the work of Donaldson, Hatai, Jackson, Slonaker, Lowrey,

King, and others. Owing perhaps to their diet and very favorable environment, most of the animals (especially the younger male groups) are somewhat larger than the average ordinary albino rats at corresponding ages. Twenty-nine litters were used but where litters contained less than four rats of a sex these individuals were usually rejected. A few rats were killed by the mother before weaning time. In all, 59 females and 73 males were fed and of these all of the females and 59 of the males were carefully autopsied at the termination of the experiment. While the number of observations is not large from the statistical point of view, and more would be necessary for final conclusions, it is believed that the present data are sufficient to establish certain points with a considerable degree of probability, and to furnish valuable evidence upon other points requiring further data. Most of the older animals which were born during the summer and early fall were smaller at the beginning of the experiment than were those born in the winter and spring. This is in accordance with previous observations. These initial differences generally persisted throughout the period of experiment (as likewise found by King ('15). Of the older litters, two of which were of purely local stock were quite large rats, whereas three litters which were of pure 'Wistar' stock were considerably smaller. Other litters used were of a mixture of these two strains.

The rats with few exceptions were weaned at 3 weeks of age, and kept in a well ventilated room in fairly large wire net cages, with wire net bottoms which allowed waste matter to drop through. The males and females were of course separated. All were fed (ad libitum) upon whole wheat (Graham) bread soaked in whole milk, a diet which seems to provide abundant nourishment, as shown by the rapid growth of the animals. During the first part of the investigation the rats were fed once a day, and their water jars washed once a week. Later all of the animals were fed 3 times a day, the water jars washed daily and at all times the cages were kept clean. Each animal was given a mark of identification with picric acid and a separate growth record kept for it. In general, each rat was weighed at wean-

ing time, and each day (before feeding) for about a week, but thereafter the interval between weighings was gradually increased.

The autopsy technique employed by Jackson ('13), with a few modifications, was used. The various organs were placed in a moist chamber when taken from the animal and weighed when all had been removed. The thyroid and thymus were freed from their capsules. In the younger groups of animals the mesentery and pancreas were removed from the stomach and intestines. These cases are marked (*c*) in the tables.

All the organs were weighed in closed containers to 0.1 mgm. The skeleton was prepared by heating the body (eviscerated and skinned) at 90 degrees centigrade for 2 or 3 hours in 2 per cent aqueous 'Gold Dust' (a proprietary soap powder) solution. The skeleton (including cartilages) was cleaned, drained carefully, and weighed, and then dried for 2 weeks (in an oven heated at 88 to 90 degrees centigrade) to constant weight and weighed again. This technique gives fairly constant results.

In the final averages shown in the various tables, extreme data are in a few cases excluded. (These extreme cases were probably due either to experimental error or to abnormal variation.)

The tables show only the averages of individual data. A copy of the original observations have been filed at The Wistar Institute of Anatomy and Biology, Philadelphia, where they may be consulted by those interested.

The material for feeding was obtained every 2 weeks by the author in person, from newly-killed calves 6 to 10 weeks of age, and ground fine in a kitchen meat-grinder. Some of the substance of each kind was spread out thin and dried before an electric fan at room temperature. The material was quite dry within 5 to 10 hours of the death of the calves. It was diluted with known amounts of milk sugar for measuring. A portion of each kind was kept fresh (at from zero to 5 degrees centigrade). No constant difference in the effects produced by the fresh and dry glands was noticeable, so in grouping the

data the difference in the two conditions of the material administered was disregarded.

The rats used, were grouped to exclude so far as possible the error introduced by the racial variability. One rat of each litter (or one of each sex if both males and females of the litter were used) was kept for control, the remaining being distributed among the groups treated, in such manner that (so far as possible) each group contained individuals from every litter. There are five groups of each sex. To eliminate the 'age' factor, the data for each sex were subdivided into 'older' and 'younger' groups, as shown in the tables.

In calculating the results, two methods were used. In one the average of the percentage of the net body weight of each organ of each group of experimental animals was compared directly with the average value of that organ in the control group while in the second method a comparison was made with the Wistar reference tables as suggested by Donaldson ('15). In the latter comparison the gross body weight was used instead of the length because the weight of the organs of the controls corresponded somewhat more closely to Donaldson's Wistar norm for rats of the same gross body weight than to those of the same length. The values obtained, however, would have been practically the same in either case.

The tables published by Donaldson ('15) used here are designated as 'Donaldson's Wistar tables' or the 'Wistar norms.'

For the dosage employed see table 3.

The experiments began when the rats were weaned, at the age of about 3 weeks, excepting 15 rats which were 8 to 11 weeks old. Of these 15 rats, only 4 were autopsied. Each rat was treated on alternate days throughout the entire period of the experiment. Nine litters were given fresh glandular substance, and all others received dried glands. The material to be fed was mixed with a small amount of bread and milk and the animal kept in an individual cage until all of this had been eaten.

IV. ORIGINAL OBSERVATIONS

1. *Body as a whole (weight and length)*

a. Comparison of controls with norms. The growth of the younger groups of rats, especially the males, in this investigation varied considerably from that of the rats described by most of the previous workers for rats collected at random from a colony. It is necessary therefore to make a comparison of the animals with the norms of other investigators before discussing the effects of the ductless gland feeding. In this comparison, only the control rats will be considered directly. With the exception of certain data of the thyroid-fed groups which will be discussed later, however, there is a close agreement among the corresponding data of all the groups so that the data of the control animals represent indirectly most of the data of the entire series.

A great difference between my rats (especially the 'younger' or 'winter-born' males) and those previously described by Donaldson and Jackson is to be noticed in the rate of growth both of body length and of body weight. The albino rats described by Donaldson ('06) and by Jackson ('13 and '15) of different strain and different diet are considerably lighter in weight than are mine at corresponding ages, up to the fourth month. (See table 1 and charts 1 and 2).. The selected 'strong and vigorous' litters described by King ('15), however, correspond rather closely with mine in body weight.

The rate of growth varies thus in albino rats from different sources, depending partly upon the 'strain,' but more upon diet and general environment. King's results indicate also that a more rapid growth may be expected from those litters in which at birth the individuals are especially large and strong. The vigorous average growth of my rats appears chiefly in those designated as the 'younger' ('winter-born') litters. The growth of the 'older' ('summer-born') animals throughout is more nearly like that found by Donaldson and Jackson (charts 1 and 2; table 1). As the 'younger' group had been eliminated largely at 110 (and partly at 70) days, the final averages are relatively

TABLE 1

Average gross body-weight of normal albino rats at various ages (in comparison with data from Donaldson, Jackson and King), showing variability probably due to various causes.

AGE DAYS	HOSKINS (CONTROLS)	KING ('15)[1]	JACKSON ('13)	DONALDSON ('06)
	grams	*grams*	*grams*	*grams*
20–21	(11m)[2] 32.6	(50m) 32.0	(53m) 24.0	(19m) 21.2
	(10f) 29.6	(50f) 28.0	(59f) 21.5	(17f) 22.6
30–31	(18m) 46.4	(50m) 48.5		(19m) 31.8
	(16f) 44.4	(50f) 45.7		(17f) 32.9
40	(18m) 69.5			
	(16f) 70.1			
42–43	(18m) 80.9	(50m) 78.0	(45m) 63.7	(19m) 46.3
	(16f) 76.4	(50f) 70.0	(50f) 64.3	(11f) 47.9
70	(18m) 164.2	(50m) 143.0	(23m) 130.4	(19m) 106.6
	(16f) 127.3	(50f) 123.0	(25f) 108.9	(11f) 99.8
90	(15m) 184.6	(50m) 184.8		
	(13f) 143.3	(39f) 148.0		
110–112	(11m) 205.9	(50m) 214.0		(19m) 183.8
	(10f) 151.6	(42f) 166.0		(11f) 160.2
150	(5m) 221.5[3]	(50m) 243.0	(20m) 167.5	(19m) 225.4
	(8f) 164.6	(45f) 185.0	(21f) 142.1	(11f) 184.6

[1] King's data partly from her table 3, partly estimated from her chart 3.
[2] The 21 day group includes only the larger 'winter-born' rats.
[3] The 150 day group includes only the smaller 'summer-born' group.

lower. At 150 days (table 1) the averages are slightly less than Donaldson's and King's, but somewhat greater than Jackson's.

As shown in table 1, the females at six weeks (40 days) average heavier than the males in the control group. This agrees with the results of Donaldson ('06) and Jackson ('13), but not with King ('15).

The difference in the rate of growth of the body length and tail length of my rats, as compared with the data of Jackson ('15) and of Donaldson's Wistar tables is shown in table 2. It may be seen that my rats averaging 13 weeks old are longer than Jackson's rats at 5 to 13 months. Had the table included my entire autopsied series of 59 males and 59 females the difference would have been slightly greater, as seen in tables 6 and 7. The ratio between the tail length and the body length is dif-

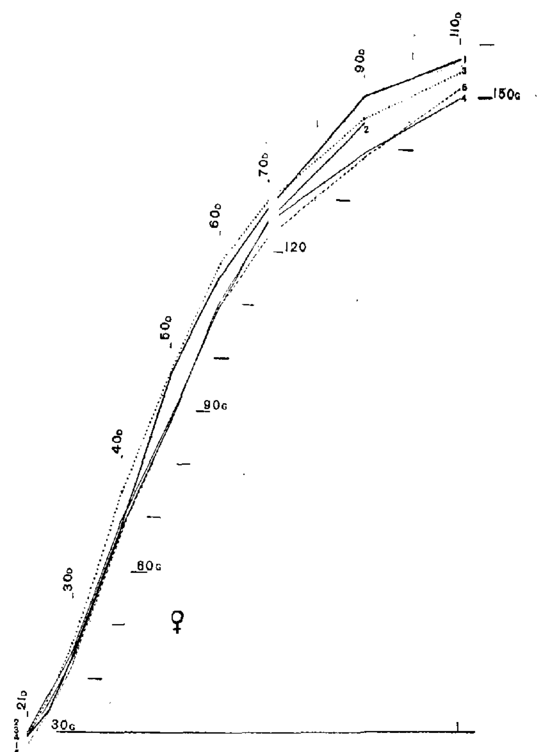

Chart 1 A graphic comparison of the data of various investigators with those of Hoskins for the growth of normal female albino rats. The weight in grams is plotted against the age in days. *D.*, Donaldson '06, *J.*, Jackson '13; *K.*, King '15; *H.*, Hoskins' older (low dosage) group of control female rats and H^1, Hoskins' younger (high dosage) group of control female rats. The sudden flattening of the graphs H^1 and H after 70 and 90 days respectively is due largely to the fact that at these points autopsy of rats was begun. The curves H^1 and H are not closely comparable with the others after these two points, because in most cases the largest rats were killed first.

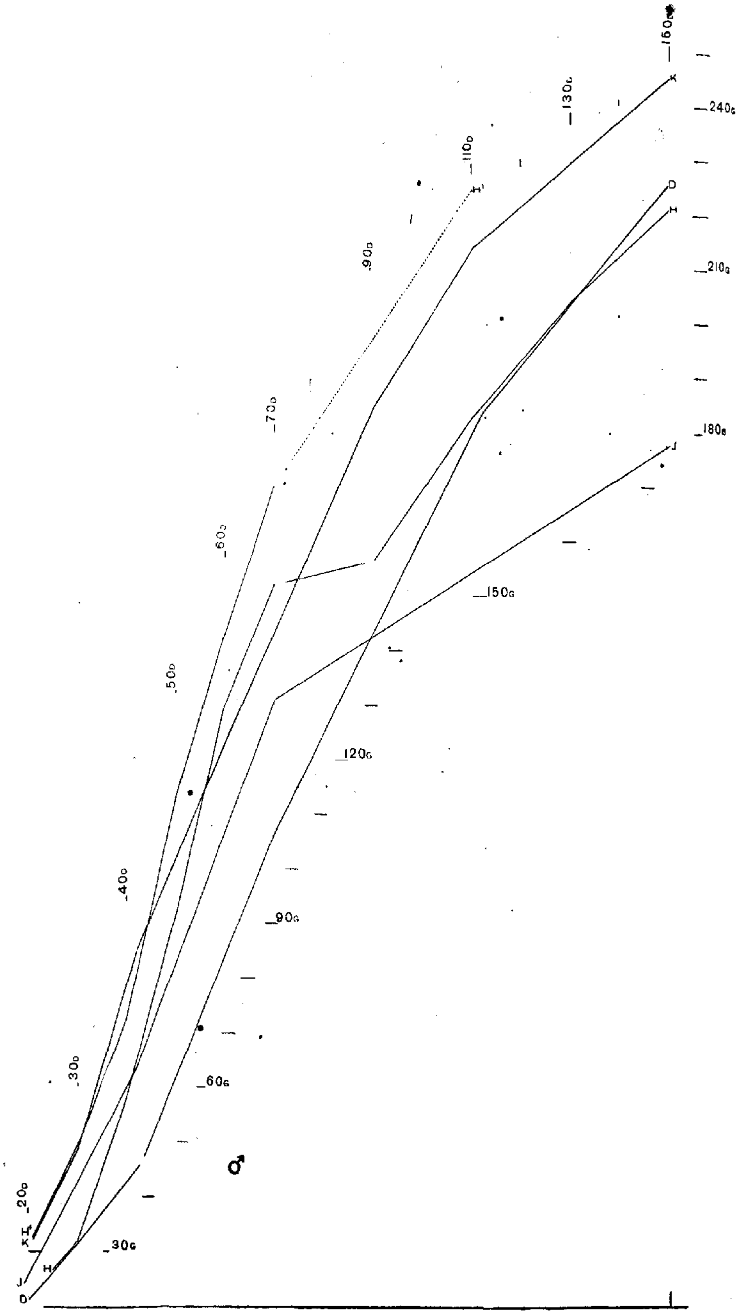

Chart 2 The same as in Chart 1, except that it is for normal males. For further explanation, see that given for chart 1.

TABLE 2

Comparison of data for younger and older groups of control (muscle-fed) albino rats with Jackson's data and Donaldson's tables, showing growth of body length and tail length.

	HOSKINS				JACKSON ('15) 5-13 MO.		DONALDSON'S TABLES (AT CORRESPONDING BODY LENGTHS)	
	Younger group (av. 13 weeks)		Older group (av. 28 weeks)					
Number and sex.....	8F*	10M*	8F.	6M.	34F.	16M.	F.	M.
Body length, cm.....	18.7	20.3	19.4	21.2	18.3	19.0	18.7-19.4	20.3-21.2
Tail length, cm......	16.0	16.5	16.5	16.6	16.8	16.2	16.4-17.0	17.2-18.0
Tail Body- Ratio....	0.86	0.81	0.85	0.78	0.90	0.86	0.88	0.85

* If all the thirteen weeks old rats on experiment are included, the number of females and males changes to 28 and 29 respectively, and the lengths of the body and of the tail are but very slightly changed. (See tables 7 and 8.)

ferent in the different series. In all of my groups of rats the tails are relatively shorter than those of Jackson's rats, and than those at corresponding body lengths in Donaldson's tables. If, instead of the age, the body weight is taken for the basis of comparison, a similar difference in the ratio of the tail length to the body length of the two series is evident.

The growth of the albino rat in weight and length under different circumstances thus varies considerably. A norm must therefore be established not only for each strain but also for each litter under a given set of environmental conditions. If, however, similar litters from the same strain are kept under similar conditions variability will be at a minimum.

For a comparison of the various organs and parts of the rats with those described by previous writers, data are shown in tables 8 and 9 for relative (percentage) weights, and in tables 4, 5, 6 and 7 for absolute weights. Data from Donaldson's Wistar tables for rats of corresponding body weights and lengths are included in tables 4 and 5. The body weight in general is slightly greater in the Wistar norms than in my rats of corresponding body length, excepting the younger males. As to the individual organs, it is evident that in some cases the weights in my series are nearer to those of Donaldson's tables at corresponding body lengths, while in others they are nearer those of corresponding body weight. Some differences are due probably

to age. On the whole, the correspondence with the Wistar tables is as close as could be expected. The individual organs will be considered later.

b. Effects of thyroid, thymus, hypophysis and pineal feeding. The weight and length of the growing albino rats fed various ductless glands are shown in tables 6 and 7, and (for the 'higher dosage' groups) in charts 3 and 4. For the sake of elimination of any variation that might be due to the age of the rats, each sex group was subdivided into 2 smaller ('old' and 'young') groups, depending upon the age of the individuals. Some of the younger animals received fresh and some dried glands (see 'Material and Methods'), but no difference was noticed in the effects produced by the two forms.

The effect of a ductless gland diet upon the growth of the females is seen in table 6 and chart 3. The various experimental groups may be compared with each other or with the controls, and it is found that the difference in weight at every age is remarkably slight. At the beginning of the experiment when the rats were 3 weeks old the different groups averaged nearly the same in weight, excepting the male thyroid group and the pineal groups. Into these groups were purposely placed slightly more than their share of smaller animals because it has recently been claimed that thyroid and pineal substances accelerate growth in various species (Schäfer '12) (Dana and Berkeley '13, McCord '14). At 70 days of age, when the period of most rapid growth had ended, it is seen that among the younger ('higher dosage') animals there had been a remarkably small difference in the growth rate of the various groups. The same is true of the older rats, if the thyroid group (which contains 2 rats that were not healthy) is left out of consideration. At 90 days of age the weights of the different groups still remain fairly close together. After 70 days the groups are no longer directly comparable because many of the rats had been killed. Upon the comparison of individuals within each litter, no constant difference appears, although considerable variation is shown. The only probable conclusion to be drawn is that the glandular substances (in

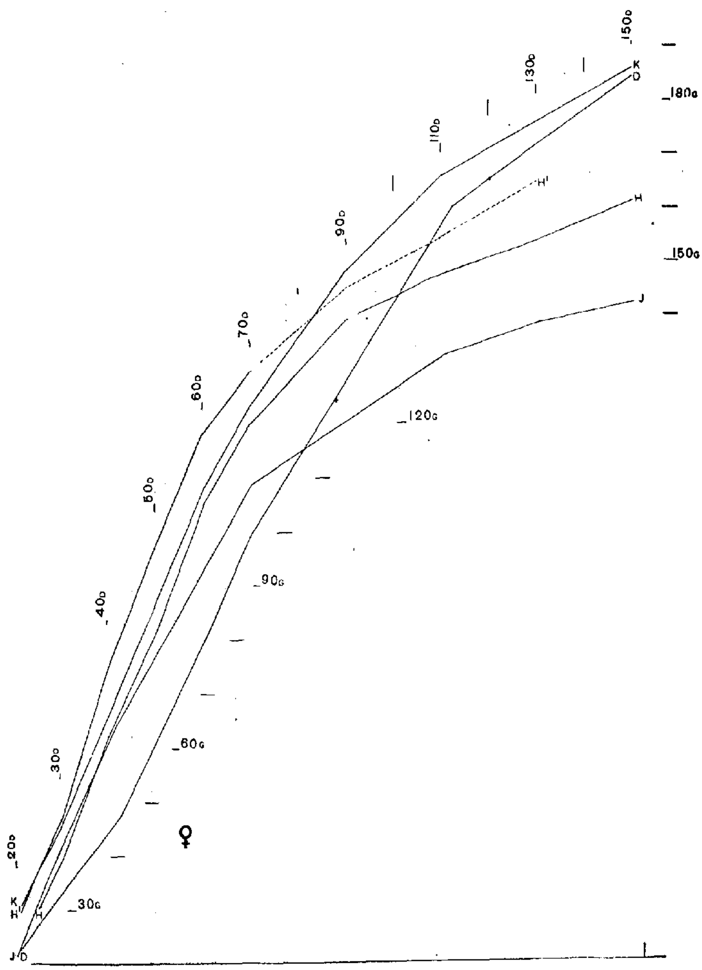

Chart 3 Graphic representation of the growth of the 'younger' or 'high dosage' female albino rats to which ductless glands were fed. The weight in grams is plotted against the age in days. *1*, Thyroid-fed; *2*, Thymus-fed; *3*, Muscle-fed (controls; see also chart 1); *4*, Hypophysis-fed; *5*, Pineal-fed. Note how closely the various groups agree in weight. At 70 days, autopsy of the largest rats was begun, hence the greater variation after this point.

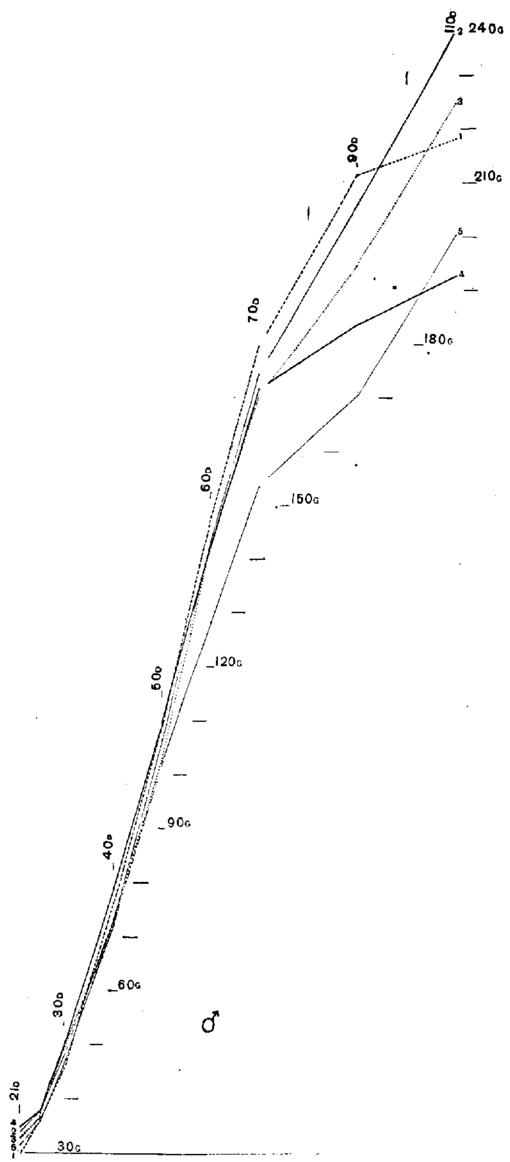

Chart 4 The same as chart 3, except that it is for males. The flattening of the graphs between 21 and 25 days (as compared with those of the females, chart 3) possibly indicates that males are more susceptible to environmental changes than are females. The rats were weaned at 21 days.

the amounts fed) had no effect upon the weight of the female albino rats in this experiment.

A graphic representation of the growth of the 'higher dosage' group of females is shown in chart 3. The body weights in all cases remain close to those of the controls (muscle-fed). In the 'higher dosage' groups, the body weights (chart 1) are seen to be much higher than the normals of Jackson and also of Donaldson (excepting near the end of the experiment). In the 'lower dosage' groups the body weights average lower, more nearly comparable to the normals of Jackson and Donaldson. This difference in body weight between the 'higher dosage' groups and the 'lower dosage' groups is not due to the different amounts of ductless glands fed, however, as a similar difference is shown by the controls in each group. A careful study of the growth of individuals within each litter shows that in nearly every instance those rats which at three weeks were larger (or smaller) than the controls retained the same relative position as regards body weight throughout the experiment.

The male albino rats to which ductless glands were fed also seemed not to be affected in body weight by the treatment. A careful study of table 7 and chart 4 shows nearly the same facts for the male rats as have just been stated for the females. The weights of the different 'higher dosage' groups are unusually close together at 70 days of age, except in the case of the pineal-fed. The 'higher dosage' pineal-fed animals at this age are 16.5 grams lighter in weight than the controls; but this difference is not great, and as this group averaged less in weight than the controls at the beginning of the experiment, the difference in weight between the two is probably due to normal variation. In the 'lower dosage' group, the pineal-fed are slightly above the controls in weight. After 70 days of age many rats were autopsied and hence the groups are no longer directly comparable, but individuals of the same litter were compared with each other and showed the same results as in the case of the females. On the whole, there appears to be no evidence indicating that the ductless gland feeding has naterially affected the body weight in any case. The differences are inconstant, and well within the limits of the variability to be expected.

It may be noted (see table 3) that thyroid was fed in varying amounts from the negligible quantity of 10 mgms. of dried gland on alternate days to a nearly maximum non-toxic dose of 200 mgms. of dried substance (or an equivalent amount of fresh gland) on alternate days. In all cases no appreciable

TABLE 3

Amount and range of dosage employed for each rat in feeding the various groups. The growth in body weight for the 'high dosage' groups is represented in charts 3 and 4.

	SIZE OF SINGLE DOSE		DOSAGE EMPLOYED FOR			
			Females		Males	
	Dried substance	Fresh substance	Average number of doses	Range	Average number of doses	Range
	mgms.	*mgms.*				
Thyroid-fed:	10	40				
'Low dosage'			4.3	4–5	1.6	0.5–2
'High dosage'			11.7	10–20	16.3	10–20
Thymus-fed:	15	70				
'Low dosage'			3.5	2–5	1.0	0.5–2
'High dosage'			16.7	10–40	16.4	10–20
Muscle-fed (controls):	8	25				
'Low dosage'			3.8	2–5	2.4	0.5–5
'High dosage'			15.8	10–40	17.0	10–30
Hypophysis-fed:	5	25				
'Low dosage'			3.5	2–5	2.3	0.5–5
'High dosage'			12.5	10–20	18.6	10–30
Pineal-fed:	7.5	40				
'Low dosage'			3.8	2–5	2.0	0.5–3
'High dosage'			17.5	10–40	13.3	10–20

effect upon the growth of the body as a whole was evident. Thymus was fed in variable amounts up to 300 mgms. of dried substance, but two individuals receiving twice this amount showed no effects different from the others. Hypophysis was administered in fairly small doses (5 to 100 mgm. of dried sub-

stance). Pineal substance was given in amounts larger than those fed to guinea pigs (in most cases) by McCord ('14), but in one of McCord's experiments where doses of 100 mgms. of dried substance was used daily, the experimental animals in a given time grew in weight 40 per cent more than the controls. As stated above, there seemed to be no difference in the result with albino rats in the present experiment, whether small amounts of 20 mgms. or larger amounts up to 150 mgms. of dried pineal substance was fed on alternate days. With the larger doses there was no evidence indicating that toxic effects of gastro-intestinal disturbances were produced by the medication.

As shown in tables 2, 6 and 7, the various rats of the same age and sex are also of nearly the same body-length regardless of treatment. The agreement here is even closer than in regard to comparative weights and emphasizes still more strongly the fact that the experimental rats as compared with the controls suffered no marked gross body changes on account of the administration of ductless glands.

The growth records of the individual rats illustrate, as has been pointed out above, that very serious errors might easily creep into the conclusions from an investigation of this kind, which includes animals from several different litters. A preliminary comparison of the individuals in each litter showed negative results, so all were finally grouped as shown in the various tables. There is always, of course, some danger in drawing conclusions from averages, but this danger is slight if the individual data are also carefully studied, and the grouping judiciously made.

Care must be taken in work of this nature to select experimental and control animals from the same litter and as nearly alike as possible. In many investigations on growth this has not been done. Experimental groups of animals of one sex have even been compared with groups of another sex; or, more commonly, an experimental group has contained both sexes in a ratio different from that in the control group.

Finally, the depressing effects upon growth and body weight obtained by some investigators by the administration of ductless glands especially the thyroid (Magnus-Levy, Bircher, Carlson, Far-

rant, Gudernatsch, Cotroni, Hewitt, Romeis, Lenhart), is in many cases possibly a general toxic effect, which is produced whenever the dosage is too high. Even a high protein diet, e.g., an excessive meat diet, may likewise be detrimental to growth, as has been shown for the albino rat by Chalmers Watson ('06).

2. Head

Data for percentage weights of head (and other organs and parts) are found for females in table 8, and for males in table 9. Absolute weights are given in tables 6 and 7.

a. Controls. The head forms an average of 9.5 per cent of the body weight in the females and 8.3 per cent in the males among my younger control rats. In the older groups, the head averages 10.3 per cent in the females, and 8.4 per cent in the males. These results are in general somewhat lower than those obtained by Jackson ('13, '15).

b. Thyroid group. The head averages very slightly heavier in both females and males to which thyroid was fed. The difference is probably insignificant.

c. Thymus, hypophysis and pineal groups. The head in these groups shows no constant variation from the controls in either direction. The few small differences are probably not significant.

3. Eviscerated Body (Tables 6 to 9)

a. Controls. The eviscerated body forms an average of 80 to 84 per cent of the net body weight in both males and females. This part of the body contains the muscles, skin, skeleton, body-fat, great vessels, lymph nodes, and spinal cord.

b. Thyroid group. The eviscerated body in all rats of both sexes (excepting 3 old males, in which the dosage was slight) is about 4 per cent less in relative (percentage) weight than that of the controls. This loss is due probably to loss of fat, which is a well-known effect of thyroid-feeding, especially with high dosage. A comparison of the body weights and body lengths in the control (muscle-fed) and thyroid groups shows a

slight relative decrease in weight in the thyroid males of higher dosage, but not in the females.

c. Thymus, hypophysis and pineal groups. In all these groups, the weight of the eviscerated body is very close to that of the controls (muscle-fed). The variations are slight and probably insignificant.

4. Integument (tables 6 to 9)

a. Controls. The average relative (percentage) weight of the integument is relatively fairly close in the various groups of both sexes. In general, the percentages range between 21 and 24 per cent of the net body weight, the value for the male rats being slightly greater than for the females, due possibly to the presence of a greater amount of fat in the latter. This is somewhat higher than that observed by Jackson and Lowrey ('12), by more than 4 per cent of the entire net body weight, and is 2 per cent of the entire body weight higher than that observed by Jackson ('15). These differences are probably due largely to the varying amount of fat (or muscle) present in the integument.

b. Thyroid group. In the thyroid rats of each sex the integument appears usually very slightly lighter in weight than in the controls, owing probably to loss of fat. Jackson ('15) has shown that during inanition the skin loses greatly in weight (probably due chiefly to loss of fat) but that the relative (percentage) weight remains unchanged in adults.

c. Thymus, hypophysis and pineal groups. In these groups, the differences in weight of the integument, as compared within each group and with the controls (muscle-fed), are well within the limits of normal variation.

5. Cartilaginous skeleton (tables 6 to 9)

a. Controls. The relative weight of the 'wet' cartilaginous skeleton averages about 7.6 per cent of the body weight in the older females, and about 6.6 per cent in the older males. The skeleton in the younger females averages about 6.8 per cent of

the net body weight and in the younger males about 6.1 per cent. These correspond fairly well with the estimate of Jackson ('15) which was 7 per cent of the adult net body weight, and with Conrow's observations (cited by Donaldson '15, table 53). The difference between the sexes is accounted for by the heavier body weight (with correspondingly lighter skeleton) in the male groups, rather than by any true sexual difference.

The older groups of each sex on the average appear to have a relatively heavier skeleton than the younger. This is contrary to the general tendency of the skeleton during growth to lag behind in relative weight. In these groups, however, the differences in body weight are much less than usual for the corresponding age differences, and the increased weight in the older skeletons is possibly due to more advanced stages of ossification and calcification. Thus in two animals of the same body weight, the older apparently has a heavier skeleton. This tendency is not evident in Conrow's data (cited by Donaldson '15), however.

In this connection may be cited the observations of Jackson ('15) who found that in young rats held at constant body weight by underfeeding the skeleton continues its development (differentiation and increase in wet and dry weights). It is therefore probable that the relative weight of the skeleton depends somewhat upon the age factor, as skeletal growth is to some extent independent of the general growth of the body.

The dry cartilaginous skeleton is likewise relatively slightly heavier in the female (3.7 to 4.6 per cent) than in the male (3.2 to 3.8 per cent), and the older rats of both sexes have relatively heavier dry skeletons than the younger. The explanation for this is doubtless the same as that above given for similar relations in the weights of the wet skeleton in different groups. There is considerable variation shown by individuals, so that the average values for both wet and dry skeleton can be considered as only approximate. Differences in the technique may also modify the skeletal weight considerably. For example, Conrow's data for the dry skeleton (cited in Donaldson's table 53) are too high, because her method of drying at room tempera-

ture is inadequate to remove all the moisture. More data are needed to establish a satisfactory norm.

b. Thyroid groups. The skeleton appears slightly heavier for the thyroid treatment in the average of the younger groups. A comparison of the individual data shows that both wet and dry skeletons average somewhat heavier in most of the rats receiving higher dosage of thyroid than in those of low dosage and controls. This suggests that the thyroid treatment may perhaps tend to stimulate skeletal development, as claimed by Bircher ('10b). However, the possibility of errors from accidental variations must be kept in mind, as the differences found are not very great, and were not constant in every litter.

c. Thymus, hypophysis, and pineal groups. The various groups fed thymus, hypophysis and pineal glands show no important or constant variation from the controls in regard to the skeleton. If any effect was produced, it is so slight as to be masked by normal variation.

6. Brain (tables 4 to 10)

a. Controls. As shown by tables 4 and 5, the absolute weight of the brain does not vary greatly from that shown by Donaldson's tables for rats of similar sex and body weight or body length.

In percentage weights, as might be expected, the males have relatively lighter brains than the females, and the older brains average lighter than the younger, on account of the fact that the brain lags behind in the growth of the body.

b. Thyroid groups. On comparing directly the average relative (percentage) weights (tables 8 and 9) of the controls (muscle-fed) and thyroid-fed animals, the brain in the latter appears slightly heavier in all except the younger group of males; but on comparing the absolute weights with the Wistar norms for animals of corresponding body weight, it is found that the difference in the various litters is not constant and the brain of the thyroid-fed rats averages even smaller than the normal (table 10).

c. Thymus, hypophysis, and pineal groups. There is no constant variation of the brain in these groups. There appears to be a slight tendency to increase in the brains of the hypophysis-fed group (less marked than in the case of the thyroid), but the difference is not constant.

7. Eyeballs (tables 4 to 10)

a. Controls. The absolute weight of the eyeballs in my animals corresponds fairly closely to that of Donaldson's norms (excepting the younger males), as shown in tables 4 and 5. In comparing the relative weights of groups of each sex (tables 8 and 9) the eyeballs appear relatively heavier in the older groups, although in these, with heavier body weight, the eyeballs would be expected to be relatively lighter. As suggested by Jackson

TABLE 4

Comparison of principal data for control female albino rats with the Wistar tables of Donaldson ('15). Weight is the average expressed in grams. Comparison is made with Wistar norms both of the same body length and of the same gross body weight.

	HOSKINS (OLDER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WT.)	HOSKINS (YOUNGER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WEIGHT)
Body length, cm	19.4	19.4	18.9	18.7	18.7	18.5
Gross body weight, g.	171.8	188.5	172.6	159.8	166.6	160.8
Tail-Ratio	0.85	0.88		0.86	0.88	
Age (days)	213			92		
Organs:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Brain	1.781	1.823	1.801	1.782	1.791	1.782
Eyeballs	0.276	0.263	0.252	0.223	0.248	0.244
Heart	0.737	0.765	0.715	0.694	0.696	0.677
Kidneys	1.379	1.622	1.503	1.589	1.458	1.414
Liver	7.530	9.600	9.000	7.405	8.770	8.540
Spleen	0.751	0.505	0.465	0.611	0.450	0.435
Lungs	1.000	1.119	1.040	0.982	1.010	0.981
Alimentary Tract	6.966	9.680	9.120			
Ovaries	0.053	0.049	0.048	0.064	0.048	0.047
Hypophysis	0.011	0.013	0.012	0.010	0.011	0.011
Suprarenals	0.049	0.050	0.046	0.049	0.045	0.044
Thymus*	(0.134)	(0.153)		(0.264)	(0.278)	

* Thymus compared with regard to age (213 days average for the older group, and 92 days for the younger group) instead of regard to body length or weight.

TABLE 5

Comparison of principal data for control male albino rats with the Wistar Tables of Donaldson ('15). Weight is the average expressed in grams. Comparison is made with the Wistar norms of the same body length and of the same gross body weight.

	HOSKINS (OLDER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WEIGHT)	HOSKINS (YOUNGER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WEIGHT)
Body length, cm.	21.2	21.2	21.1	20.3	20.3	20.6
Gross body weight, g.	232.5	238.1	234.1	214.9	204.3	215.0
Tail-Ratio	0.783	0.85	0.85	0.813	0.85	0.85
Age (days)	193			90		
Organs:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Brain	1.852	1.911	1.907	1.909	1.872	1.885
Eyeballs	0.286	0.294	0.291	0.220	0.273	0.280
Heart	0.893	0.918	0.905	0.988	0.814	0.847
Kidneys	1.867	1.992	1.962	2.050	1.740	1.820
Liver	10.701	11.440	11.290	10.915	10.200	10.590
Spleen	0.959	0.630	0.620	0.895	0.545	0.572
Lungs	1.813	1.365	1.346	1.199	1.198	1.251
Alimentary Tract	9.351	11.360	11.230			
Testes	2.222	2.397	2.375	2.248	2.203	2.267
Hypophysis	0.009	0.009	0.008	0.008	0.008	0.008
Suprarenals	0.031	0.038	0.038	0.031	0.035	0.036
Thymus*	(0.155)	(0.170)		(0.298)	(0.283)	

* Thymus compared with regard to age (193 days average for older group, and 90 days average for younger group) instead of regard to body length or weight.

('13), however, the growth of the eyeballs may tend to be correlated with age, rather than with body weight (as is known to occur in the thymus). These differences may therefore be due to age changes, to normal variability, or perhaps merely to differences in the technique of removal of the eyeballs.

b. Thyroid groups. The eyeballs average heavier in relative weight than those of the controls in both sexes, but the differences obtained are slight and probably of no significance. Comparison according to the method of Donaldson ('15) shows them slightly lighter in weight in the males than those of the corresponding controls, but in the females slightly heavier.

c. Thymus, hypophysis and pineal groups. In most cases, the eyeballs in these groups average relatively slightly heavier than those of the controls. No particular significance other than variability is attached to this fact.

8. *Thyroid Gland* (*tables 6 to 9*)

a. Controls. The thyroid of my rats cannot be compared directly with that of other investigators owing to the different technique with which it was removed from the body, as described in 'Material and Methods.' The weight is about one-third less than the Wistar norm, probably on account of removal of the capsule. There is also extreme variation in the weight of this gland even in different members of the same litter. Usually it appears relatively heavier in the older than in the younger rats, and also slightly heavier in females. Jackson '(13) and Hatai ('13) also have found the thyroid gland to be exceedingly variable, so no final conclusions can be drawn as to the normal weight of this gland.

b. Thyroid groups. The thyroid gland (tables 6 to 9) shows no constant changes as a result of the thyroid feeding. Any effect if produced is hidden by the great normal variability. In view of the great variability in the weight of this organ, final conclusions in regard to the effect of thyroid feeding upon the weight of the thyroid gland are not justified from the available observations.

c. Thymus, hypophysis, and pineal groups. In these groups likewise the thyroid appears variable when compared with the controls, and the results are not sufficiently marked or constant to warrant any conclusion regarding the effects upon the weight of the thyroid gland of feeding these substances.

9. *Thymus* (*tables 4 to 9*)

a. Controls. The thymus also is not to be compared very closely with that described by previous workers. The usual method of comparison considers the weight of the gland at different ages. Theoretically, the organ in the albino rat increases in size gradually until at about 85 days it reaches its maximum weight of 0.29 grams (Hatai '14), but as is well known, many conditions influence the involution of this organ. Its weight varies considerably in rats of the same size and age even when the animals are apparently normal and kept under nearly

Females: Average absolute weight (in grams) of parts in albino rats grouped according to diet and age when autopsied

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	5	4	5	6	8	8	6	5	7	5
Average age (days)	207	80	215	88	213	92	196	92	214	95
Gross body weight, g.	160.2	145.3	158.0	156.3	171.9	159.9	166.1	149.6	173.3	146.2
Net body weight, g.	169.7	140.2	151.3	149.1	165.4	152.9	151.8	142.4	164.7	138.3
Body length, cm	19.3	18.4	18.9	18.5	19.4	18.7	18.8	18.6	19.5	18.3
Tail length, cm.	15.9	16.0	16.4	15.6	16.5	16.0	15.3	16.0	15.9	15.6
Average number doses	8.6	12.5	11.7	16.7	11.6	16.3	12.8	12.0	11.9	20.0
Average weight of:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Head	15.6	13.4	15.8	13.9	16.3	1.41	15.6	14.4	16.3	14.0
Eviscerated body	127.6	125.7	123.5	135.8	148.5	128.7	128.4	126.4	143.9	117.9
Integument	31.5	32.5	32.4	33.9	36.3	31.7	31.2	32.0	35.3	29.2
Wet cartilaginous skeleton	11.24	9.95	10.88	7.94	11.18	10.39	11.94	10.22	12.05	10.40
Dry cartilaginous skeleton	6.69	5.49	6.63	5.23	7.47	5.51	7.02	5.64	7.332	5.623
Brain	1.714	1.723	1.683	1.742	1.781	1.782	1.714	1.806	1.858	1.538
Eyeballs	0.276	0.211	0.275	0.177	0.276	0.223	0.276	0.228	0.282	2.27
Thyroid	0.017	0.14	0.014	0.018	0.017	0.015	0.0197	0.0151	0.018	0.017
Thymus	0.120	0.232	0.074	0.221	0.134	0.264	0.125	0.220	0.114	0.212
Heart	0.850	0.761	0.672	0.656	0.737	0.694	0.663	0.676	0.696	0.611
Lungs	1.917	0.851	1.588	0.945	1.900	0.982	2.047	0.911	1.929	1.208
Liver	9.383	9.266	6.943	8.567	7.530	7.405	6.941	7.484	7.180	8.188
Spleen	0.910	0.633	0.794	0.537	0.751	0.611	0.633	0.534	0.502	0.659
Stomach-intestines (empty)	7.445	3.172[1]	6.528	3.137	6.966	2.962[1]	7.191	3.244[1]	7.568	3.690
Suprarenals	0.0647	0.0525	0.0393	0.0454	0.0485	0.0486	0.0493	0.0548	0.0464	0.0395
Kidneys	1.916	1.829	1.620	1.574	1.379	1.589	1.289	1.641	1.485	1.337
Ovaries	0.496	0.652	0.436	0.614	0.053	0.064	0.530	0.615	0.455	0.492
Pineal	0.0011	0.0012	0.0011	0.0012	0.0011	0.0011	0.0012	0.0011	0.0011	0.0012
Hypophysis	0.0093	0.0088	0.0101	0.0088	0.0111	0.0100	0.0101	0.0094	0.0098	0.0084

[1] Freed of mesenteries and pancreas.

TABLE 7

Males: Average absolute weight (in grams) of parts of albino rats grouped according to diet and age when autopsied

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	7	4	5	4	6	10	6	7	6	4
Average age (days)	164	94	162	94	193	90	188	84	202	87
Gross body weight	210.5	209.5	225.6	231.9	235.0	214.9	228.9	208.7	233.6	192.7
Net body weight	198.9	199.3	215.0	223.8	225.3	206.3	215.4	197.5	227.3	184.8
Body length, cm	21.0	20.7	21.2	20.7	21.2	20.3	21.0	19.7	21.2	19.4
Tail length, cm	17.0	17.3	16.8	17.2	16.6	16.5	16.8	16.9	17.0	15.9
Average number doses	10.8	15.0	11.7	17.5	10.0	17.0	10.3	20.7	7.4	15.0
Average weight of:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Head	17.2	16.9	17.9	17.6	18.8	17.3	18.7	16.0	18.9	15.3
Eviscerated body		166.4	169.8	191.9	184.9	171.6	177.6	156.7	205.2	159.5
Integument	43.3	46.2	47.6	52.0	51.3	47.2	46.2	53.7	51.9	43.4
Wet cartilaginous skeleton	13.36	12.42	15.43	13.10	14.62	12.77	13.67	11.43	14.13	14.37
Dry cartilaginous skeleton	7.93	6.82	8.72	7.20	8.59	6.70	8.44	6.02	8.66	6.07
Brain	1.839	1.850	1.872	1.887	1.852	1.909	1.846	1.800	1.802	1.729
Eyeballs	0.269	0.226	0.283	0.225	0.281	0.220	0.277	0.210	0.227	0.208
Thyroid	0.021	0.014	0.023	0.016	0.028	0.016	0.023	0.020	0.023	0.021
Thymus	0.164	0.298	0.220	0.333	0.155	0.298	0.153	0.323	0.139	0.225
Heart	1.060	1.119	0.988	0.957	0.893	0.988	0.896	0.851	0.857	0.824
Lungs	1.768	1.522	1.996	1.458	1.665	1.199	1.954	1.091	1.008	1.402
Liver	11.563	12.183	10.182	11.063	10.701	10.991	10.991	10.763	11.999	9.208
Spleen	1.241	0.997	0.796	0.559	0.958	0.895	0.883	0.868	0.709	0.717
Stomach-intestines (empty)	9.496	3.179[1]	9.533	3.740[1]	9.351	4.097[1]	8.392	3.408[1]	9.479	3.941[1]
Suprarenals	0.0361	0.0469	0.0340	0.0356	0.0306	0.0311	0.0281	0.0309	0.0302	0.0263
Kidneys	2.223	2.387	1.894	2.086	1.867	2.050	1.891	1.979	2.162	1.676
Testes	2.148	2.422	2.121	2.274	2.222	2.248	2.047	1.903	2.450	2.031
Epididymi	0.748	0.644	0.794	0.624	0.753	0.613	0.672	0.547	0.736	0.539
Pineal	0.0013	0.0018	0.0015	0.0013	0.0014	0.0014	0.0014	0.0012	0.0012	0.0014
Hypophysis	0.0086	0.0090	0.0081	0.0083	0.0085	0.0079	0.0082	0.0073	0.0078	0.007

[1] Freed of mesenteries and pancreas.

TABLE 8

Females: Average relative (percentage of net body) weight of parts in albino rats grouped according to diet and age when autopsied

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	5	4	5	6	8	8	6	5	7	5
Average age (days)	207	80.5	215.4	87.5	212.6	91.7	195.6	91.8	213.7	95.4
Average net body weight g.	169.7	140.2	151.3	149.1	165.4	152.9	151.8	142.4	164.7	138.0
Average number dose	8.6	12.5	11.4	16.7	11.6	16.8	12.8	12.0	11.9	20.0
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Head	10.56	9.64	10.61	9.34	10.28	9.54	10.28	10.13	10.00	10.02
Eviscerated body	79.26	80.87	82.08	83.15	83.72	82.51	82.44	83.69	83.33	82.02
Integument	21.04	22.95	21.36	22.86	21.75	22.43	20.89	22.55	21.35	21.15
Wet cartilaginous skeleton	7.53	7.12	7.31	6.86	7.57	6.81	7.61	7.15	7.44	7.31
Dry cartilaginous skeleton	4.46	3.92	4.45	3.53	4.59	3.69	4.45	4.00	4.47	3.93
Brain	1.16	1.26	1.14	1.19	1.10	1.17	1.14	1.27	1.05	1.23
Eyeballs	0.187	0.152	0.186	0.149	0.171	0.141	0.188	0.162	0.172	0.164
Thyroid	0.011	0.010	0.010	0.012	0.011	0.010	0.013	0.010	0.011	0.012
Thymus	0.080	0.161	0.061	0.157	0.070	0.172	0.079	0.184	0.071	0.154
Heart	0.576	0.546	0.446	0.444	0.449	0.455	4.43	0.477	0.428	0.442
Lungs	1.31	0.61	1.03	0.62	1.22	0.64	1.40	0.64	1.04	0.87
Liver	6.24	5.98	4.63	5.71	4.58	4.89	4.13	5.30	4.36	5.87
Spleen	0.604	0.473	0.530	0.361	0.478	0.400	0.418	0.373	0.438	.481
Stomach-intestines (empty)	4.96	2.32[1]	4.59	2.49[1]	4.24	2.07[1]	4.79	2.36[1]	4.62	2.73[1]
Suprarenals	0.043	0.037	0.026	0.031	0.029	0.031	0.030	0.035	0.029	0.028
Kidneys	1.30	1.32	1.06	1.06	0.92	1.04	1.05	1.09	0.91	1.05
Ovaries	0.032	0.047	0.029	0.041	0.032	0.043	0.034	0.039	0.029	0.036
Pineal	0.00075	0.00084	0.00073	0.00084	0.00067	0.00069	0.00080	0.00078	0.00075	0.00082
Hypophysis	0.0062	0.0063	0.0068	0.0062	0.0067	0.0065	0.0067	0.0067	0.0059	0.0061

[1] Freed of mesenteries and pancreas.

TABLE 9

Males: Average relative (percentage of net body) weight of parts in rats grouped according to diet and age when killed

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	7	4	5	4	6	10	6	7	6	4
Average age (days)	164	93.8	161.6	94.3	192.9	90.0	188.1	84	202.1	87
Average net body weight g.	198.9	200.3	215.0	227.5	225.3	206.3	215.4	197.5	227.0	184.8
Average number doses	10.8	15.0	11.7	17.5	10.0	17.0	10.3	20.7	7.4	15.0
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Head	7.37	8.44	8.38	7.89	8.40	8.34	8.84	8.13	8.46	7.89
Eviscerated body	83.1	80.74	82.85	84.24	82.24	83.20	81.81	80.07	83.46	83.52
Integument	21.33	22.86	22.05	22.95	22.48	23.02	21.65	23.35	21.94	23.51
Wet cartilaginous skeleton	6.52	6.23	7.03	5.90	6.57	6.13	6.20	5.97	6.24	6.35
Dry cartilaginous skeleton	4.02[2]	3.39	4.09	3.31	3.83	3.21	3.89	3.06	3.96	3.28
Brain	0.93	0.93	0.88	0.86	0.84	0.94	0.88	0.91	0.80	0.94
Eyeballs	0.129	0.114	0.130	0.102	0.119	0.110	0.122	0.110	0.110	0.113
Thyroid	0.010	0.007	0.011	0.007	0.013	0.008	0.011	0.010	0.012	0.011
Thymus	0.73	0.155	0.104	0.153	0.072	0.051	0.072	0.167	0.62	0.167
Heart	0.545	0.555	0.465	0.486	0.401	0.472	0.425	0.435	0.435	0.446
Lungs	0.89	0.76	0.94	0.65	0.74	0.60	0.92	0.55	1.01	0.79
Liver	5.88	6.16	4.76	5.04	4.87	5.16	5.23	5.47	4.13	4.95
Spleen	0.634	0.516	0.398	0.257	0.453	0.443	0.437	0.439	0.315	0.419
Stomach-intestines (empty)	4.95	2.23[1]	4.43	1.88[1]	4.18	2.19	4.05	2.07[1]	4.05	2.14[1]
Suprarenals	0.019	0.024	0.016	0.016	0.014	0.015	0.013	0.016	0.014	0.016
Kidneys	1.13	1.18	0.89	0.94	0.91	1.05	0.89	1.01	0.82	0.90
Testes	1.08	1.20	0.98	1.06	1.01	1.09	0.98	0.96	0.95	1.09
Epididymi	0.38	0.31	0.37	0.28	0.34	0.36	0.32	0.28	0.35	0.29
Pineal	0.0065	0.00088	0.00070	0.00056	0.00062	0.00069	0.00065	0.00061	0.00052	0.00076
Hypophysis	0.044	0.0045	0.0038	0.0037	0.0038	0.0039	0.0037	0.0037	0.0034	0.0040

[1] Freed of mesenteries and pancreas.
[2] Group contains 1 rat with an exceptionally heavy skeleton.

identical conditions. According to Jackson ('13) the coefficient of variability in weight of the thymus at 10 weeks is 22 to 25.

When compared in absolute weight with Donaldson's Wistar norm for rats of corresponding age (tables 4 and 5) it appears that in my rats the thymus is somewhat lighter, except in the younger male group. The age involution is well known on comparing the older and younger groups (tables 6 to 9), the corresponding body weights in both cases being not greatly different.

b. Thyroid groups. The thymus shows no constant difference in weight that can be attributed to the thyroid diet. It is under weight in the females by an average of 10 per cent, but not in the males.

c. Thymus, hypophysis, and pineal groups. The thymus appears relatively much heavier than normal in the thymus-fed older males, but as no corresponding difference is found in the younger males, or in the females, the result is probably due to accidental variations.

10. Heart (tables 4 to 10)

a. Controls. In absolute weight, the heart of the older rats in my series (tables 4 and 5) is in general in fairly close agreement with the corresponding data in Donaldson's tables, but in my younger males the heart is considerably heavier than Donaldson's norm for rats of corresponding body weight or length. Jackson ('13) also found the normal heart relatively somewhat heavier than would be expected according to Hatai's curve of theoretical growth.

b. Thyroid groups. The heart shows a very marked hypertrophy in rats to which thyroid was fed, excepting 3 old males, in which the dosage was too small to be effective. If calculated by Donaldson's method, the hypertrophy amounts to 24.6 and 16.7 per cent for the older and younger females respectively, and 36 and 15.4 per cent for the corresponding males (table 10).

c. Thymus, hypophysis, and pineal groups. No constant or apparently significant variations appear in the heart in these groups.

TABLE 10

Thyroid-fed albino rats. Average percentage deviation of organ weight from that of the control rats, compared according to Donaldson's method ('The Rat,' Donaldson '15).

ORGAN	OLDER GROUPS		YOUNGER GROUPS	
	Females	Males	Females	Males
	per cent	*per cent*	*per cent*	*per cent*
Brain	− 3.6	+ 0.1	+ 0.5	− 5.5
Eyeballs	+ 0.5	− 0.1	+ 6.8	− 8.5
Heart	+24.6	+36.0	+16.7	+15.4
Liver	+26.7	+24.6	+30.5	+ 6.4
Spleen	+41.3	+86.0	+15.0	+ 6.4
Alimentary canal	+12.1	+11.8		
Suprarenals	+16.1	+38.1	+14.5	+36.4
Kidneys	+46.3	+44.4	+33.0	+40.4
Ovaries	− 3.2		− 3.5	
Testes		− 0.2		+ 9.8
Hypophysis	−11.5	+21.3	− 8.8	+18.0

11. Lungs (tables 4 to 9)

a. Controls. The weights of the lungs in the older rats are considerably heavier than those of Donaldson's tables, owing to the prevalence of pulmonary infection in my older rats. The younger rats are in close agreement with the Wistar tables.

b. Thyroid groups. On account of the great variation due to the frequency of lung infection (especially in the older animals), no conclusions can be drawn from the apparent changes in the weight of the lungs in the thyroid-fed groups.

c. Thymus, hypophysis, and pineal groups. There are no differences that are of significance in the weights of the lungs in these groups.

12. Liver (tables 4 to 10)

a. Controls. In absolute weight, the liver averaged somewhat below the corresponding figures in Donaldson's Wistar tables, excepting in the younger males. Jackson ('13) also found the liver normally considerably below the theoretical curve derived by Hatai ('13). The liver is known to vary greatly under different circumstances, however, and is considerably

influenced in size by diet, etc. One might expect a relatively larger liver in the female, which is said to be more active than the male (Slonaker '12), since exercise has been shown to cause hypertrophy of this organ (Hatai '15). Jackson ('13) found the liver heavier in the male, however, and this is apparently true also for my control animals.

b. Thyroid groups. In the thyroid-fed groups, (tables 8 to 10) the liver appears relatively considerably heavier than in the controls. When compared according to Donaldson's method, the females show an increase in the absolute weight of the liver of 26.7 per cent and 30.5 per cent for the older and younger groups respectively, and the males show a corresponding increase of 24.4 per cent and 6.4 per cent. The apparently small increase in the weight of the liver of the younger males is probably to be explained by the fact that the liver is unusually heavy in the corresponding control group. Even when due allowance for normal variability is made, a hypertrophy of the liver due to thyroid feeding is therefore strongly indicated.

c. Thymus, hypophysis, and pineal groups. In all the younger groups of females the liver averages larger than in the controls, but as no corresponding difference is found in the other groups, the variation is probably not significant.

13. Spleen (tables 4 to 10)

a. Controls. As appears by comparing the individual data, the absolute weight of the spleen in the control groups is from 30 per cent to 100 per cent higher than the figures for corresponding animals in Donaldson's Wistar tables. In the latter tables, however, the figures for the spleen are derived from the formula of Hatai ('13) who used data from which the 'enlarged' spleens had been excluded. Whether these tables for the spleen really represent the true norm is therefore questionable, since it is not known whether the 'enlarged' spleens are actually pathological, or represent merely extreme cases of normal variation in size. Jackson ('13) found the variability

of the spleen in the rat very high (coefficient of variation averaged about 35). His relative (percentage) values for the normal spleen (including all specimens) are also somewhat above the curve derived from Hatai's formula (excluding enlarged specimens), but are considerably lower than mine, excepting the old male group (tables 8 and 9). It is evident that my control spleens are relatively larger than that which is usually considered normal, but it is impossible to say whether or not this is due to normal variability. A study of individual data shows that the weight of the spleen in most rats varies in the same direction as that of the liver. Dr. Hatai of the Wistar Institute has told me that he also has noted such correlation. This fact is in agreement with the doctrine that the spleen functions in furnishing certain materials to the liver for use in general metabolism. Sweet and Ellis ('15) state that where digestion is interfered with greatly by removal of the external function of the pancreas, the spleen undergoes marked simple atrophy.

b. Thyroid groups (tables 6 to 10). In relative weight where the averages of the thyroid groups are compared with the entire control group it is seen that there is an increase in the weight of the spleen of about 25 per cent. According to Donaldson's method of comparison the increase in absolute weight amounts to 41.3 per cent and 15 per cent in the older and younger female groups, and of 86 per cent and 6.4 per cent in the corresponding male groups (table 10). The apparently small increase shown by the younger group of males is due to an unusually large spleen in the control group, in which the liver was also unusually large (as above mentioned).

c. Thymus, hypophysis, and pineal groups. As might be expected, the spleen in these groups shows considerable variability, but it is probably within the limits of normal variation. There appear decidedly smaller spleens in the thymus-fed male group, but the results are not constant, and of doubtful significance.

14. Alimentary canal (tables 4 to 10)

a. Controls. As may be seen in tables 4 and 5, the absolute weights obtained for the alimentary canal (empty stomach and intestines, plus mesentery and pancreas) average slightly lower than in Donaldson's tables corresponding to the older males and females. In my younger rats, no data for this system are available. The alimentary canal, including the stomach and intestines when freed from the pancreas and mesentery, weighs about 3 grams in the younger female rats (average 13 weeks old) and 4.1 grams in the males of the same age, forming about 2.1 per cent of the net body weight in the former and a little less in the latter (tables 8 and 9). The measurements can be made only approximately on account of the difficulty in removing completely the contents of the canal without loss of a part of the mucosa. No data for comparison with the digestive canal without mesentery are available.

b. Thyroid groups. The empty alimentary canal appears heavier in relative weight both in males and females of the older thyroid groups than in the corresponding controls (tables 8 and 9). In no case is the difference very large, however, and owing to variability and difficulty in securing exact weights of the empty canal, the difference is of doubtful significance. Compared by Donaldson's method, the increase in weight is about 12 per cent in each older group (table 10).

c. Thymus, hypophysis, and pineal groups. The variations observed in the alimentary canal of these groups (tables 6 to 9) in comparison with the controls are inconstant, and probably within the limits of normal variability and experimental error.

15. Suprarenal glands (tables 4 to 10)

a. Controls. In absolute weight as compared with Donaldson's norms, the suprarenals of my rats (tables 4 and 5) are somewhat light in the case of the males, but correspond more closely in the females.

In relative weight (tables 8 and 9) my data correspond fairly well with the results of Jackson ('13), but the suprarenals of

my rats have a slightly higher percentage in the females and lower in the males, than in Jackson's older rats of corresponding body weight. The sexual difference in the weight of the suprarenals discovered independently by Jackson ('13) and by Hatai ('13) occurs likewise in my rats.

b. Thyroid groups. As shown in tables 6 to 10, the thyroid-fed animals show a distinct increase in the relative size of the suprarenals in all groups. According to Donaldson's method of comparison, the younger females show an overgrowth in the absolute weight of the suprarenals of 14.5 per cent, the older females of 16.1 per cent, the younger males of 36.4 per cent, and the older males of 38.1 per cent (table 10). This indicates that the reaction of the suprarenals to thyroid treatment is relatively greater in males, in which sex the gland is normally relatively lighter in weight than it is in the females.

c. Thymus, hypophysis, and pineal groups. In all these groups the suprarenal glands do not appear to differ from the controls more than might be expected from the normal variability (tables 6 to 9). In the younger pineal-fed female rats, however, the suprarenals average about 9 per cent lighter in weight than in the corresponding controls.

16. Kidneys (tables 4 to 10)

a. Controls. As compared with Donaldson's tables, the kidneys in my rats (tables 4 and 5) were somewhat heavier in the younger groups and lighter in the older groups. The differences are not great, however, and may possibly be due to age. My younger rats are larger than is usual at that age. The relative size of the kidney tends to decrease with the less active metabolism of adult life.

In relative (percentage) weight (tables 8 and 9), all the groups are in fairly close agreement with the results obtained by Jackson ('13) for rats of the same age. In two rats of the same sex and same size but of different age, the younger usually has larger kidneys.

b. Thyroid groups. The kidneys reacted to the thyroid feeding in a manner similar to that described for the liver, but to a greater extent (tables 6 to 10). According to Donaldson's method of comparison the increase in the absolute weight of the kidneys is as follows: older females 46.3 per cent, younger females 33.0 per cent, older males 44.4 per cent, and younger males 40.4 per cent (table 10).

c. Thymus, hypophysis and pineal groups. As shown in tables 6 to 9, the kidneys in the various groups fed on thymus, hypophysis and pineal gland show no very constant variations from the controls. The differences are mostly small and are probably due to normal variability. The kidneys of the hypophysis-fed females average 12 per cent heavier than the controls, however, and those of the pineal-fed males average 16 per cent under normal weight.

17. Ovaries (tables 4, 6, 8 and 10)

a. Controls. In absolute weight, the ovaries appear considerably heavier for the younger group, and slightly heavier for the older group (table 4) than the corresponding ovaries in Donaldson's tables. In relative (percentage) weight, the ovary in the younger groups similarly exceeds the normal found by Jackson ('13), and is about equal in the older group. The ovaries are normally found to be exceedingly variable in weight. This is to be explained partly on account of varying stages of ovulation, and partly to technical difficulties of dissection (Jackson '13).

b. Thyroid groups. The ovaries in the thyroid groups appear nearly unchanged, the slight differences being very probably due to normal variation.

c. Thymus, hypophysis, and pineal groups. On comparing the relative weights of the ovaries in these groups, no important or constant changes are found (table 8), except in the pineal-fed group. In these the ovaries appear considerably under weight, as compared with the controls. The decrease in absolute weight shown by Donaldson's method is 16.7 and 27.0

per cent for the older and younger groups respectively. Possibly pineal feeding retards growth of ovaries, but on account of their great variability and the comparatively small number of observations, there is considerable doubt as to whether the decrease noted is significant.

18. Testes (tables 5, 7, 9 and 10)

a. Controls. In absolute weight, compared with Donaldson's tables, the testes in my rats average slightly lighter (table 5)

In relative (percentage) weight (table 9), in comparison with the observations of Jackson ('13) on animals of corresponding weight, the testes in my series appear relatively lighter in the older group and of about the same relative weight in the younger groups. Jackson's data included the epididymis, however, and on this basis, with testis and epididymis combined, both my old and young groups would appear to have heavier organs.

b. Thyroid group. The testes of both sub-groups of the thyroid-fed males (table 9) show a slight increase in relative weight as compared with the entire group of controls. This overgrowth ranges from about 7 per cent in the group of older rats (in part of which the dosage is very small and ineffective)to 13 per cent in the young animals. Calculated by Donaldson's method the older rats are seen to have testes of the same absolute weight as the controls, and the younger rats to have heavier (10 per cent) testes than the corresponding controls. As in the case of the ovaries, however, the testes show considerable normal variability, and conclusions based upon a relatively small number of observations must be carefully guarded.

c. Thymus, hypophysis, and pineal groups. In these groups the testes show no important or constant changes in comparison with the controls, and the differences (e.g., the apparent slight retardation of the testis in pineal-fed) are probably not significant. Microscopic examination of the testis of a thymus-fed rat showed no degeneration, although Hewer ('14) found such condition in rats, some of which were fed but slightly more thymus than some of my rats received. (Mine were fed up to 1.4 grams fresh or 0.15 grams dried thymus on alternate days; hers were fed 1 to 4 grams of the fresh thymus daily.)

19. Epididymi (tables 7 and 9)

a. Controls. Few data are available for comparison. Jackson ('13) states that after the age of puberty the epididymis weighs about one-third as much as the testis, and in extreme cases one-half. In my series (tables 7 and 9) the eipdidymi average about one-fourth as heavy as the testes in the younger (0.28 per cent of net body weight), and about one-third as heavy in the older group (0.34 per cent of net body weight). Some of the variability in the weight of the epididymis is due to the difficulty in removing the fat associated with it.

b. Thyroid groups. The epididymi in the thyroid group, like the testes, appear slightly larger than in the control rats (tables 7 and 9). It is doubtful whether they are stimulated in growth by the thyroid treatment, however.

c. Thymus, hypophysis, and pineal groups. In these groups, as was true in regard to the testis, the variations in comparison with the controls are probably not significant.

20. Pineal body (tables 6 to 9)

a. Controls. No attempt has hitherto been made to determine the normal weight of this small organ in a large series of albino rats. Biedl ('13) gives the weight as 0.0020 g., which is considerably higher than the average weights in my series (0.0011 g. in the females and 0.0013–0.0014 g. in the males, tables 6 and 7). The weight values obtained vary greatly on account of the extremely small size of the gland. Relatively (tables 8 and 9) there seems to be no significant difference according to sex or age, although the percentage weight appears to be somewhat less in the animals of greater body weight, and this to some extent is due to age. Recently, from data secured through the kindness of Dr. Donaldson and Dr. Hatai, I note that the values obtained for the pineal body in rats at the Wistar Institute agree fairly closely with mine.

b. Thyroid groups. The pineal body in the thryoid-fed rats (tables 8 and 9) averages in relative weight somewhat heavier in the various groups than in the corresponding controls. The

difference is not constant in every litter so that, although it is possible that the thyroid medication increases the weight of this organ, the data are insufficient for a final conclusion.

c. Thymus, hypophysis, and pineal groups. On comparing the relative weights of the pineal body in these groups, the results in the case of the thymus-fed and hypophysis-fed are very inconstant. In the pineal-fed, as in the thyroid-fed rats, the pineal body appears usually increased in relative weight. The difference in weight is not constant in every litter, and hence is of doubtful significance, in all of these three groups.

21. Hypophysis (tables 7 to 10)

a. Controls. In absolute weight the hypophysis in both male and female rats in my series agree fairly well with Donaldson's tables for rats of corresponding body length or body weight (tables 4 and 5). There is a sexual difference in the weight of this organ, as discovered by Hatai ('13). As seen in tables 8 and 9, the average percentage of the net body weight for the hypophysis is about 0.0066 per cent in the females and 0.0039 per cent in the males (not much change according to age or body weight). Thus the hypophysis, like the suprarenal body, is in the adult rats relatively much larger in the female.

b. Thyroid groups. On comparing the thyroid-fed animals with the controls (tables 8 to 10), there appears a very slight decrease in relative weight of the hypophysis in the female groups. In both male groups there is a decided increase in the weight of the hypophysis. With Donaldson's method of comparison the decrease in absolute weight in the older and younger female groups is 11.5 and 8.8 per cent respectively, and the increase in the corresponding males is 21.3 and 18.0 per cent (table 10). These results may be interpreted as indicating a sexual difference in the effect of thyroid feeding upon the hypophysis, the effect being most marked in the males. The thyroid-feeding therefore tends to reduce the normal difference between the sexes in the weight of the hypophysis.

22. *Summary*

a. Comparison of controls with established norms for body weight, body and tail length, and size of organs. In regard to body weight, the control rats used in this investigation as well as the experimental animals can be divided into two groups: (1) an older group ('summer-born') which were born in the summer and early fall; and (2) a younger group ('winter-born') which were born in the winter and spring. The first group maintained about the same size and body weight as the rats of Donaldson ('06) and Jackson ('13). The second group, especially the males, were considerably larger at every stage, and more nearly resembled in growth the selected strong and vigorous litters of rats recently studied by King ('15).

The ratio of tail length to body length is somewhat less in my series than in the norms of Jackson ('15) and those calculated from Donaldson's Wistar tables. That is, my rats were relatively short-tailed.

The variability of growth in body weight, body length and tail length is thus emphasized. Such differences may occur in different 'strains' of rats, especially under different conditions of environment, diet, etc. Experience shows that the average body weight may be considerably increased by unusual care and liberal feeding. Exercise has also been shown to affect markedly the growth of the body (Slonaker '12) and organs (Hatai '15) of the rat.

As to the absolute weights of the individual organs and parts, the data for the controls in general agree fairly well with those of Donaldson's Wistar tables for corresponding body length and body weight (tables 4 and 5). The differences are in most instances no greater than might be expected from normal variability, especially in rats of different strain kept under different environment. In some cases (e.g., thyroid gland, thymus, and alimentary canal) there are differences due to difference in the technique of removing the organs.

In the case of the very rapidly growing rats, it is quite probable that some of the peculiarities (e.g., of skeleton, spleen, liver,

heart, kidneys) in comparison with older rats of similar body weight may be due to changes with age, independent of body weight. The younger rats with more rapid metabolism may have relatively larger organs. Donaldson has found a close correlation between the water content of the central nervous system and age in the rat. The age involution of the thymus is well known, and Jackson ('13) found indications of correlation with age in the weight of the eyeballs. Similar relations are possible in other organs, although organ weight in general is doubtless more closely correlated with body weight than with age.

In relative (percentage) weights, the organs likewise are in general correspondence with the results of Jackson ('15), the differences in most cases being within the limits of normal variability to be expected. There was noted a marked correlation between the weights of the spleen and liver in most cases.

b. Effects of thyroid feeding. The thyroid diet apparently affected the gross body weight of the rats but very slightly. The 'higher dosage' animals averaged slightly heavier than the controls and other groups (charts 3 and 4), but the difference is perhaps too slight to be significant. If the loss in fat of the thyroid-fed animals be taken into account, an increased weight of the remainder of the body appears.

The results are not incompatible with the view that by thyroid feeding with small dosage an increase in body weight is produced (as found by Moussu '99, and Schäfer '12), while with higher dosage a decrease or retardation in body weight is produced (Magnus-Levy '95; Moussu '99, Bircher '10; Carlson, Rooke and McKie '12; Gudernatsch '12, '14, '15; Hewitt' 14; Cotroni '14; Romeis '15). The decrease or retardation of body weight reported by most of the above mentioned investigators is possibly due to the toxic effect of large doses. I observed that two thyroid-fed rats apparently in good health were killed by a single large dose (200 mgm.) of dried thyroid substance.

The eviscerated body and (to a slighter extent) the integument of the thyroid-fed fats usually show a slight loss in relative weight, probably due to loss of fat.

The organs of the rats which received very little thyroid were not much affected in weight, but in the rats which received larger doses (see tables 6 to 10) several of the organs are evidently hypertrophied. This cannot be interpreted as merely a relative gain, due to loss of body-fat, for the body weight, as above stated, has apparently suffered no loss in the thyroid-fed rats, but possibly a slight gain instead. The organs which show a considerable increase in weight are the heart, liver, spleen, suprarenals and kidneys.

Other organs in which the results are more doubtful, but which seem to be increased in weight to a greater or lesser extent are the hypophysis (male), and alimentary canal, and possibly the skeleton, testes, and epididymi. The increase of this group of organs is uncertain, and in some cases probably due to chance variations.

These results are in general agreement with those of Iscovesco ('13) who (by thyroid extract injections) found hypertrophy of the heart, thyroid gland, suprarenals, kidneys (male), testes, ovaries and uterus. He reports an increase in the weight of these organs of nearly 100 per cent, but no increase in the weight of the liver or female kidneys. My results confirm also the hypertrophy of the suprarenal glands found by Rudinger, Falta and Eppinger ('08), and by Hoskins ('10a). They also confirm to some extent the results of Bircher ('10a) (10b), who found that hyperthyroidism produces enlargement of the heart and thyroid gland, and acceleration of skeletal development. They do not, however, agree with Utterström ('10), who found enlargement of the thymus.

It may be noted further that these results likewise agree with the generally accepted doctrine that hyperthyroidism produces a general acceleration of metabolism. Conditions increasing metabolism would naturally throw a greater burden upon the important viscera, and thus tend to produce in them a hypertrophy. Thus Hatai ('15) has shown that increased exercise in albino rats tends to produce an enlargement of various organs, and his results resemble closely those obtained by me with thyroid feeding.

c. Effects of thymus feeding. In general, the results of thymus feeding were negative. Neither the body as a whole nor any of the individual parts or organs showed any marked or constant apparent effect, in comparison with the controls. The apparent tendency to decrease in the relative weight of the male spleen is a doubtful exception.

The negative results are in agreement with those of Miss Hewer ('14), except as to the degeneration of the testis; but they do not confirm in the rat the stimulating effect upon body growth obtained by Gudernatsch ('12) ('14) and (in part) by Romeis ('15) in amphibian larvae.

d. Effects of hypophysis feeding. The data in the present experiments show that the weights of the body as a whole and of the various parts and organs of the albino rats in the hypophysis-fed groups were even more closely in agreement with the controls than were the thymus groups. The apparent increase in weight shown by the kidneys and thyroid of the females is probably not significant.

The negative results of the hypophysis feeding in these experiments confirm similar findings as to negative effects upon body weight by Caselli ('00); Sandri (anterior lobe)('09); Hoskins ('11); Aldrich ('12a) ('12b); Schäfer ('12); Lewis and Miller ('13), and Gudernatsch ('14). On the other hand they do not agree with Schäfer ('09) and Goetsch ('16) who from very few data reported growth stimulated by feeding anterior lobe of hypophysis, nor with the following who obtained retardation of growth (especially skeletal) by hypophysis feeding: Thompson and Johnston ('05); Etienne and Parisot ('08); Sandri ('07) ('09) (posterior lobe); Cushing and Goetsch (cited by Cushing '12); Wulzen ('14), and Pearl ('16) (anterior lobe). My results, likewise disagree with Hallion and Alquier ('08) who obtained hypertrophy of the suprarenal glands, and with Wulzen ('14) who found accelerated involution of the thymus. It may be noted, however, that my dosage was comparatively small subtoxic, and this might account for the negative results.

e. Effects of pineal feeding. The pineal groups also agree fairly closely in weight with the control animals, both as to the

body as a whole and in the various organs and parts. Several minor differences are to be noted, but all are perhaps within the limit of normal variability. The testes, kidneys (male), ovaries, and suprarenals show a decrease in weight which is of doubtful significance, as (except in the case of the ovaries) it is not constant in every litter. My results agree with Priore ('15) who obtained negative results on body growth by injection of pineal extracts. They disagree, however, with those of Dana and Berkeley ('14) and McCord ('14, '15) who obtained an acceleration in body growth by feeding pineal substance.

V. CONCLUSIONS

Some of the principal results of the present investigation may be summed up briefly as follows:

1. The normal growth of the albino rat varies materially, not only in different 'strains' and under different conditions of environment, but even among litters when all conditions are as nearly constant as possible. It is therefore not sufficient to rely upon the established norms of growth for comparison, but in all cases experimental and control animals should be selected of the same sex and from the same litter or litters.

2. In the case of rats with unusually rapid (or slow) growth, in addition to comparison with (older or younger) rats of corresponding size and weight, due regard for possible changes correlated with age must be observed. Such changes have previously been noted in the water content of nervous system (Donaldson), weight of the thymus (Hatai), and probably the weight of the eyeballs (Jackson). Indications of similar age-changes (independent of body weight) are found in the skeleton, liver, kidneys, heart and spleen. Compared with animals of the usual age at a given body weight, the skeleton seems to be relatively heavy in older animals, and the other organs are apparently heavier in younger rapidly growing animals.

3. There is apparently no sexual difference in the relative weight of the pineal body, such as has been found in the suprarenal glands (Jackson '13, Hatai '13) and the hypophysis (Hatai '13).

4. Thyroid feeding (in sub-toxic doses) causes little or no change in body weight in growing rats. There is possibly a slight stimulation in growth of the body as a whole, balanced by a decrease in the amount of free fat in the body. There is a slight loss in the relative weights of the eviscerated body and the integument, probably due to loss of body-fat.

5. Thyroid feeding produces a decided hypertrophy of the heart, liver, spleen, kidneys and suprarenal glands (especially in males). It apparently causes also a somewhat less extensive and more uncertain increase in the weights of the alimentary canal and hypophysis (male) and possibly in the skeleton, testes and epididymi, and a decrease in the weight of the hypophysis of females.

6. Thymus feeding (with the dosage employed) has no apparent effect upon the growth rate of the body of albino rats. No constant or important effect upon any of the individual organs or parts was observed. The testis showed no degenerative or other changes.

7. Hypophysis feeding (with the sub-toxic dosage employed) produces no marked or constant effect upon the growth rate of the body or organs of albino rats.

8. Pineal feeding likewise produces no apparent changes in the weight of the body or organs of the albino rat, beyond differences probably within the limits of normal variation.

VI. LITERATURE CITED

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ON THE BLASTOLYTIC ORIGIN OF THE 'INDEPENDENT' LENSES OF SOME TERATOPHTHALMIC EMBRYOS AND ITS SIGNIFICANCE FOR THE NORMAL DEVELOPMENT OF THE LENS IN VERTEBRATES[1]

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TWO TEXT FIGURES AND TWO PLATES

The problem of the development and differentiation—dependent or independent—of the lens of the vertebrate eye has in recent years formed the subject of many interesting investigations.

In 1901 Spemann attempted its solution by experiments in which the anlage of an optic cup in the neurula-stage of Rana fusca was mechanically destroyed. From the failure of many larvae to develop a lens on the side operated upon he concluded that the lens of the vertebrate eye depends for its development upon a contact stimulus from the optic cup. Herbst ('01) concluded from the fact that in higher vertebrates the development of the lens never begins before the optic vesicle has come into contact with the ectoderm from which it arises that the latter is essential and that the development of the lens is to be regarded as a 'specific' (chemical?) 'thigmomorphosis.' As evidence for the justification of this hypothesis he adduced the cyclopean monsters, i.e., embryos with a single median eye. In such embryos, he reasoned, no lenses develop on the sides of the head, that is, in the usual position of the eyes, because the ectoderm of these areas, owing to the absence of lateral optic vesicles, does not receive the specific thigmomorphotic stimulus necessary for its differentiation into lenses. On the other hand, he concluded, since a lens does develop in association with the

[1] Aided by a grant from the Carnegie Institution of Washington.

median eye from ectoderm which normally does not give rise to a lens, it is evident that the heterotopic (cyclopean) optic vesicle furnishes the formative stimulus for its differentiation from indifferent ectoderm.

Soon, however, the validity of this exceedingly suggestive hypothesis, became questionable, for Mencl ('03) described two lateral lenses in an anophthalmic head (without any traces of optic cups) of an anadidymus in Salmo salar. From these findings he concluded that no stimulus from the optic cup was necessary for the differentiation of the lens. More recently Mencl ('08) has recorded free lenses in several anophthalmic Salmo embryos.

In the meantime the problem had again been attacked experimentally by Barfurth ('02) W. H. Lewis ('04), King ('05), Le Cron ('07) and Spemann ('07). The evidence which Barfurth was able to bring forward for the 'dependent' differentiation of the lens in the chick was rather inconclusive. Of a very decisive character, however, seemed to be the results which Lewis obtained in his experiments on Rana palustris. He removed the optic vesicle of tadpoles with the least possible injury to the overlying ectoderm which was raised from it and reflected forward. No lenses developed in such embryos on the side operated on and Lewis' conclusions fully confirm Herbst's views. Only a year later, however, King experimenting on embryos of the same species by destroying the optic vesicle with a heated needle, was able to show that lens buds developed on the eyeless side of the embryo which might perhaps have fully differentiated into lenses, had the embryos been kept alive for a sufficient length of time. This remarkable discrepancy of results increased from now on with practically every attempt at the solution of the problem. Le Cron, experimenting on Amblystoma was able to confirm Lewis' important findings, but Spemann ('07) found that in Rana esculenta a lens may be formed without any stimulus from an optic vesicle or cup. In the next year Mencl was able to confirm the observations he had recorded in 1903, and in 1909 Stockard employing a chemical method, succeeded in producing in Fundulus experimentally many terato-

phthalmic monsters in which the free lens was of rather frequent occurrence. From his observations which strongly corroborate Mencl's statements, Stockard concluded that the lens is capable of development without any stimulus from the optic cup.

Mencl's and Stockard's results and their conclusions seemed to be very convincing, and, on the whole, the major evidence seemed to be in favor of the independent origin of the lens by 'self-differentiation.' The climax of the difficulty, however, had not yet been reached, for, in 1912 Spemann reported on the basis of very extensive experiments that the same methods of operation on the same stages of frog larvae of two species belonging to the same genus yielded conflicting results, the one species being capable of forming lenses without the stimulus of an optic cup, while in the other species lenses were not formed if the optic vesicle had been removed.

What is the cause of this remarkable incongruity of apparently correct observations? Why is Amblystoma incapable of forming a lens independently of the optic cup, while in Rana esculenta this ability apparently exists? Why is Rana palustris capable of differentiating a lens without the contact stimulus from an optic cup, when the optic vesicle is destroyed by a heated needle and incapable of doing so when the latter is removed by cutting? Why in cyclopean Fundulus embryos does the single median optic vesicle stimulate by contact the formation of a lens from ectoderm which would normally not differentiate into a lens, while in other teratophthalmic, and particularly, anophthalmic embryos of the same species free lenses frequently occur without any trace of an optic cup? And, finally, why should two species of the same genus differ so widely in their morphogenetic potentiality, the one (Rana esculenta) being capable of forming a lens when the optic vesicle has been removed, while in the other (Rana fusca) this apparently does not occur, after the same operation performed at the same larval stage?

The answer to these perplexing questions, I think, is that we are here dealing with an apparently morphological problem for whose solution neither our morphological methods of investigation nor mechanical methods of experimentation seem to be

fully adequate. The problem is, as I hope to make clear in the following, pre-eminently a biochemical one, as Lewis has suggested already in 1904.

During the summers of 1914 and 1915 I performed a large number of experiments on fertilized eggs of Fundulus heteroclitus by exposing the latter in early cleavage stages to some toxic products of pathologic metabolism in order to test the hypothesis that these products may underlie the 'spontaneous' origin of monsters. Among the many monsters thus produced those with terata of the eyes were found to be of most frequent occurrence. In some of the latter free lenses were found. The location of such 'independent' lenses is strikingly variable. Thus, for instance, a cyclopean embryo was once found, which had besides the lens of the median eye also a free lens, normal in size and structure, in a lateral position (Werber '15 a, fig. 28, p. 551). In a symmetrically monophthalmic embryos a lens of approximately normal size may sometimes be found on the side lacking the eye, or several small lenses may be met with on various parts of the head in a lateral or anterior position. In such and in anophthalmic embryos one or more small lenses are sometimes found on the anterior tip of the head, while in one asymmetrically monophthalmic embryo three small lenses were found on the maxilla (Werber '16 b, figs. 78 and 79). In many instances small free lenses were also observed posterior to one of the eyes in deformed embryos possessing two eyes in the usual lateral position.

The perfect analogy between these observations and those of Mencl's (l.c.) and Stockard's (l.c.) is evident. For in all these cases lenses have developed, without the contact stimulus from an optic cup, from indifferent ectoderm which would normally not have given rise to such structures. If Mencl's and Stockard's interpretation according to which these lenses are to be considered as products of self-differentiation were justified it would necessarily apply also to the free lenses which I have recorded. Yet, is the evidence of Mencl and Stockard conclusive? Will it stand the test of critical examination? I believe that I shall be able to show in the following that these questions cannot be answered in the affirmative.

It is very significant that free lenses are found only in teratophthalmic embryos. No case has yet been recorded of a free lens in the presence of two normal, perfect eyes, while such a lens may occasionally be found in the presence of both eyes if the latter exhibit some structural imperfections. And in such cases of monophthalmia asymmetrica, where the single lateral eye is in perfect structural harmony with a normal eye, a free lens, if found, is always on the side lacking the eye. Briefly it may be said that free lenses do not occur where there is no eye defect. This rule is so constant that some correlation between the eye defect and the 'independent' lens must necessarily be assumed. Granting this (and the evidence to prove just this point, is, indeed, overwhelming) it might be expected that if it were possible to determine the nature of the defect, i.e., the mechanism which causes it, we would at the same time obtain a very definite clue towards the genesis of the free lens.

I have attempted an analysis of the nature of the defect which leads to teratophthalmia, and, on the basis of very definite and constantly increasing evidence, have arrived at the conclusion that we are here dealing with a destructive process of dissociation of parts of the blastoderm. The nature of this process, which I have termed blastolysis, has been very briefly outlined in two of my previous publications (Werber '15 b and '16 a) while a more complete treatment of the subject will be found in a paper now in press (Werber '16 b) to which the reader is particularly referred.

For the sake of clearness in the following presentation it may be stated, however, that blastolysis either destroys part or all of the germ's substance or it may dissociate and disperse fragments of the latter.

A careful study of the morphology of teratophthalmic embryos has yielded unmistakable evidence that all of them are due to a defect of blastolytic nature in the anterior region of the head. In other words, the formation of terata of the eye comes about through destruction of parts intermediate to the earliest anlagen of the eyes or of parts of the latter. The most striking evidence for this blastolytic process is dispersion of fragments

of ophthalmoblastic material which sometimes develop into fragments of an optic cup. I have observed several such instances and have always found that the relation between such a teratoma and the terata of the eyes in the same embryo is clearly one of syngenesis. In several of such embryos free lenses were found which were located far away from an eye or optic cup fragment.

These observations which fully agree with Stockard's ('09, '10) and Mencl's ('08) findings,[2] and the fact mentioned above that free lenses occur only in teratophthalmic embryos suggested that the origin of such lenses is in some causal connection with blastolysis. The dispersion of ophthalmoblastic material and the simultaneous presence of free lenses in some teratophthalmic embryos suggested strongly that the origin of the latter depends upon the former. While examining sections of many teratophthalmic embryos during the winter of 1915 this hypothesis forced itself upon me by the sheer weight of the remarkable concurrence of facts, which could not, by any means, be considered, as fortuitous. For, why do free lenses arise only in such cases where the eyes are defective as a result of blastolysis, that is, where ophthalmoblastic substance has been partly destroyed, and partly dissociated or dispersed?

These considerations have, finally, led me to the following theoretical conclusion which I regard as a possible basis for more refined experiments towards the solution of the lens-problem.

It could be imagined, I thought, that just as in normal development two lateral lenses arise as the result of an apparent contact stimulus from two lateral optic cups and just as in the cyclopean eye one median lens arises as a result also of a contact stimulus of one median eye, the free lenses of teratophthalmic embryos could arise from indifferent ectoderm owing to a stimulus from a fragment of ophthalmoblastic material too small to differentiate in the state of its isolation into a morphologically discernible structure. This stimulus is, as Herbst (l.c.) has pointed out, not a contact stimulus only, but very probably a specific thigmomorphosis, the mechanism of which I am inclined

[2] Cf. Stockard ('09, fig. 45, p. 317 and '10, figs. 6 and 8, p. 403 and figs. 22, 23, 24, p. 409) and Mencl ('08, plate xx, figs. 5, 6, 7, and 8).

to consider as similar to (or possibly identical with?) an enzyme reaction. In other words it might be imagined that ophthalmoblastic substance (and most likely potential retina) when in contact with epidermis acts as a catalyzer for the chemical reactions necessary for the transformation of the latter into a lens.

Very strong support is lent to these conclusions by the results of some more recent experiments in which I have employed a modification of one of my methods. By employing weak solutions of acetone and long exposures I expected that embryos might thus result in which less destruction and more dissociation would be noted. And such was actually found to be the case.

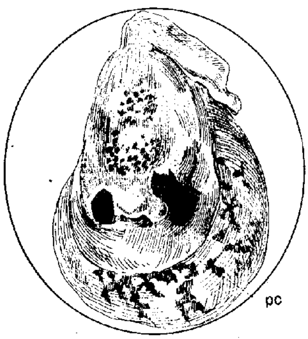
While some of the eggs so treated have given rise to synophthalmic or monophthalmic monsters, others have developed into embryos with two large, grotesquely protruding eyes in the usual lateral position in the head (fig. 2). Some of the embryos from these experiments possess two ill-formed eyes of medium size, one of which may often be far protruding, or in some cases one or both eyes may already on macroscopic examination exhibit evidence of dissociation, the pigment layer being either partly duplicated (fig. 1), or fragments of it being observable between or behind the eyes. Examination of sections shows that these eyes are elongate and ovoid in shape and it seems clear that during their formation some forces must have been acting which tended to disintegrate and dissociate the anlage from which they have developed (blastolysis).

A detailed description of the microscopic anatomy of such 'blastolytic eyes' is reserved for another publication, soon to follow. For the present it will suffice to state that in such embryos a number of free lenses of various sizes often are found in the head region on examination in toto, while each one of the eyes may possess a lens of its own in contact with the optic cup. Examination of sections of some of these embryos, however, discloses a most surprising multitude of small lentoids anterior to the eyes.

A brief description of sections through the anterior part of the head of one such embryo may now follow. Already in toto

(fig. 1) it is seen that the eyes are greatly deformed, only their pigmented parts being discernible. The right eye appears to be rather small, while the left eye is large and protruding and exhibits striking evidence of dissociation. Transverse sections (6μ in thickness) show the following conditions:

Already in the second section there can be seen at the base of the forebrain on both sides a number of small lentoid bodies,



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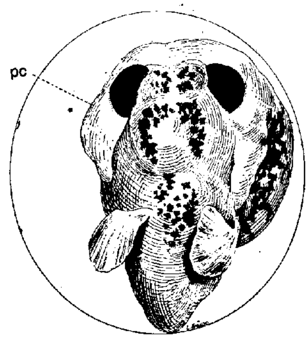

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Fig. 1 Teratophthalmic embryo, twenty-nine days old, from acetone solution (20 cc. gram molecular in 50 cc. sea water). Exposure one hundred and forty-four hours, solution renewed every day.

Fig. 2. Teratophthalmic embryo, twenty-three days old, from the same experiment as the embryo in figure 1.

which if followed in succeeding sections are found to form an almost continuous mass. In the same section one still smaller lentoid is seen on the periphery of the integument, while in further sections nearly all of the strata of some parts of the epidermis seem to be transformed into such lentoids. At this level (48μ from the tip of the head, figure 3)[3] there is seen at the base of the brain a fairly large lentoid with a number of smaller ones near by. All lentoids so far recorded in this description are in a stage of differentiation transitional between the epithelium of the lens-bud and the fibrillae of the fully developed lens. Follow-

[3] It is a pleasure to acknowledge my indebtedness to Professor Petrunkevitch for his kind assistance in making the photomicrographs.

ing the sections caudalwards, we find in the tenth section (60μ from the tip of the head) the beginning of the lens of the right eye. In the section preceding it is seen the capsule of the lens on two sides of which several small lentoids are noted. The lentoid mass at the base of the brain is no longer seen at this level; only a few very small lentoids can be observed in this position in the eleventh and twelfth sections. In the latter there appears also the first indication of the pigment layer of the left eye. Two sections more caudalwards the optic cup of the right eye begins to come into view. At this level the optic cup of the left eye can already be clearly distinguished. Its pigment layer is of a dark brown hue instead of a solid black, which is due to the fact that the pigment cells are considerably dissociated. The layer of rods and cones, while not fully differentiated, is, however, clearly discernible. All the other layers of the retina are spread out far beyond the limits of the optic cup into a continuous mass which reaches the lens of the other eye and goes all around the unusually small oral cavity. From the epithelium of the latter there arise several small lentoids which are in contact with this blastolyzed retina. Four sections more caudalwards the mouth disappears, its place being taken up entirely by six small lentoids which are partly in connection with each other through lens fibers. In this section (fig. 4) and in three more succeeding sections the dissociated pigment layer of the left eye presents a strange appearance. It bulges out on one side of the optic cup to form a very large pocket into which a part of the retina dips. This pigment 'pocket' corresponds to a part of the apparent duplicature of the pigment layer of the left eye seen in toto in figure 1. The left eye possesses no lens but its blastolyzed retina surrounds the six oral lentoids spoken of above, which in more posterior sections increase in number and form a rather dense cluster. A number of small lentoids can be observed also in the epidermis ventrally from the eyes.

The optic cup of the right eye is, as compared with its large lens, strikingly small and its tissues, while dissociated, are much less so than those of the other eye. However, the blastolytic dissociation of this eye is plainly discernible in posterior sections.

A very remarkable feature is presented by this optic cup in the twenty-eighth and twenty-ninth sections (fig. 5). Here a small body is found which has the same staining reaction as the lens of this eye and all the lentoids observed in the embryo. It is situated in proximity to the layer of rods and cones and is surrounded by practically all other layers of the retina. On first examination the impression was gained that we are here dealing with an artifact, namely a lens fragment carried in by the knife during sectioning. This suspicion, however, proved to be unfounded for the following reasons. First, the structure appears in two sections in exactly the same position, and it is larger in the second section containing it, the part observed in the preceding section being its tip. Secondly, if it were an artifact, it would necessarily have to overlie a corresponding part of the retina, which is not the case, for on deep focusing on the first part (contained in section 28) nothing can be observed below the section of this structure, while a deep focus on its second part (section 29) discloses only pigment cells, with which it is surrounded on all sides but one. The morphogenesis of this lentoid is, of course, uncertain. But it seems probable that we are here dealing with a 'retinal lentoid' such as was in many instances observed by Fischel ('02) after mechanical injury of the retina. In this case our retinal lentoid would have to be regarded as a case of embryonic heteromorphosis induced by chemical alteration of the environment.

More evidence for blastolysis is presented by the dissociated epidermis,[4] and the unusually distorted shape of the brain of this embryo as well as by the significant fact that in posterior sections of the eyes there appear dorsally from the latter two more rudimentary optic cups, the retina of which (rods and cones) while not fully differentiated, yet is clearly discernible. These optic cups have not been pushed out by the brain, but each one forms a part of its dorso-lateral wall almost exactly where one would expect the optic lobes, which latter, owing to the distortions of the brain, can nowhere be recognized. This

[4] The embryo was alive when fixed. All my observations on teratological material were made only on embryos alive at the time of fixation.

indicates that the ophthalmoblastic material of each side has been fragmented by blastolytic action of the modified environment into two parts, one of which was capable of differentiating almost as much as in normal development, while the other attained only a rather low degree of differentiation.

The longitudinal axes of both eye bulbs are dorso-ventral instead of transverse as they should be in the fish. This deviation in their position in relation to the brain is, undoubtedly, also due to blastolytic dissociation and subsequent readjustment of parts. Owing to this dissociation the optic anlagen have during their progress towards the unusual position which they finally have come to occupy, left fragments of optic material in their trail, and have in this way stimulated a number of lentoids to arise, which can be found in a number of sections, most prominently, on the right side, between the last part of the eye and the rudimentary optic cup of the same side (fig. 6) At this point (the region of fragmentation of one original eye anlage into two) a loose mass of apparently disintegrated tissue, only the nuclei of which have for the most part remained, can in a number of sections be observed to extend all the way from the rudimentary optic cup with which it is continuous into the oral cavity. The latter is at this point defective, a part of its cartilaginous roof lacking. Careful examination of sections shows that the nuclei of the disintegrated tissue mass are mainly of two sizes. The impression is gained that these nuclei are the remnants of potential retinal cells, the large ones possibly corresponding to the cells of the ganglionic layer while the small ones to those of one of the molecular layers. If we imagine that the disintegrated tissue mass was fragmented and dispersed from the earliest primordium of the eye of this side, it is conceivable that many of its cells were yet capable of farther development and have made some steps in differentiation. To the latter may be due the differences between the nuclei of this 'syncytium,' corresponding to differences between the various layers of the retina. At any rate, the tissue mass in question suggests forcibly that we are here dealing with a retinal fragment, which was largely disintegrated after some differentiation. It is only neces-

sary to compare it with sections through the anterior part of the eyes where areas of such disintegration and intermingling of 'naked' nuclei of the same two types are also found, to disarm any skepticism on this point.

If, however, it is granted that the tissue mass in question is retina, the origin of the lentoids of this region as well as in the most anterior part of the embryo can no longer be doubted as due to contact of ectoderm with dispersed particles of ophthalmoblastic substance. Blastolysis has in this way affected also the brain, which besides its unusual distortions also exhibits distinct traces of disintegration. Part of the cartilaginous roof of the mouth in this region is also lacking and it is this part that is filled with disintegrated retina. If the sections be followed caudalwards the roof of the oral cavity is observed to appear gradually more and more complete until a section is reached where the defect no longer exists. At this level, no more disintegrated retina is found in the mouth, but imbedded in the oral epithelium a number of lentoids can be observed in six sections (fig. 7). Beyond this level the epithelium of the corresponding part of the oral cavity consists in several sections of large vesicular cells which look decidedly as if they were in a stage intermediate between the epithelial and early lentoid. Of all the evidence for the blastolytic origin of the lentoids of this embryo presented so far, the lentoids of the oral cavity form, I believe, the most striking if not the most important, part. For here the lentogenic reaction (as I would term it) of ectodermal epithelium to optic cup substance is demonstrated in a manner, which would seem to leave no room for reasonable doubt.

Not less obvious is the conclusion to which these observations would seem to lead. The free lenses of teratophthalmic embryos recorded by Stockard, Mencl and myself, are not independent in their differentiation, but due to a specific (catalytic?) reaction of dispersed fragments of optic cup substance on the ectoderm with which they may chance to come in contact.

Mencl's and Stockard's observations regarding the free lenses in teratophthalmic embryos which seemed to form such important evidence against Herbst's theory of the developmental cor-

relation between optic cup and lens can now, in the light of our findings, be fully harmonized with it.

Of the 'independent' lenses recorded in the experiments of other authors, those described by King (l.c.) can easily be accounted for, when it is considered that the method employed in her experiments contains an uncontrollable source of error. For it is easy to see that of the optic vesicles of frog larvae which she destroyed, some particles might have been dispersed and come into permanent contact with the overlying epidermis in which in this manner the 'lentogenic reaction' was induced. Provided that this conclusion is correct (and I think it is highly probable that it is) it is on the other hand just as easy to understand why for the same frog species in the experiments of Lewis (l.c.) in which a more reliable method was employed, the recorded results were the opposite to those of King's. By skillful operations, Lewis removed the optic vesicle in such a manner that no fragments of it came into contact with overlying ectoderm. Therefore the 'lentogenic reaction' was never induced in the latter on the side operated upon. The same would seem to hold good for the experiments on Amblystoma performed by Le Cron (l.c.) under Lewis' direction. It follows from these considerations that the free lens buds recorded by King are in their origin similar to those of blastolytic origin in teratophthalmic embryos, the dissociation or dispersion of optic vesicle substance (histolysis) having been induced by mechanical force.

Greater difficulty is presented to our interpretation by the contradicting results which Spemann ('12) has obtained in two frog species of the same genus. However, this difficulty is not insurmountable, for I am firmly convinced that the apparent discrepancies in the effect of the same operations in species of the same genus are not due to primary differences in morphogenetic potentiality, but rather to some secondary factor, which, however, in these minutely delicate operations becomes of great importance inasmuch as it may present a source of unavoidable error similar to that which I have assumed as present in King's experiments. Spemann ('12) himself has pointed out some technical difficulties which may affect the accuracy of experimenta-

tion. Thus, for instance, he has observed that the consistency of the embryonic tissues varies with the different frog species, the substance of embryos of some species being more rigid and thus easier to operate on, while in other species it may be very sticky and offer great difficulty to mechanical separation of ectodermal epithelium from underlying cell layers. I am therefore inclined to conclude that owing to this difficulty in many experiments in which Spemann attempted the complete elimination of an eye vesicle, some fragments of the latter were left attached to the epidermis, from which they eventually stimulated the formation of lenses. Such fragments may be too minute for detection with the aid of the binocular microscope and too small to differentiate histologically; yet they may be capable of furnishing the stimulus for the lentogenic reaction, for which perhaps only a very few potential retinal cells may suffice. This might account for the development of the independent lenses in such experiments of Spemann where an optic vesicle has been removed, while in the species in which no such lenses have developed, although the same operation had been performed, it is evidently easier to separate with perfect accuracy the optic vesicle from the overlying epidermis and to remove it in such a manner that no traces of it are left. In view of Spemann's well known skill in such minute operations and his scrupulous care, we may, I think, expect that he will sooner or later be able to confirm this interpretation of the divergence in the results of these as well as other experiments (transplantation of epidermis from a posterior region of the embryo onto an eye vesicle from which the epidermis has been removed—Spemann, '12).

If it were (and perhaps it some day will be) possible to control the difficulties spoken of, it would most likely be found that there are no exceptions to the rule that the lens of the vertebrate eye cannot originate independently of a stimulus from optic cup substance. The difficulty presented to the lens-problem by Mencl's and Stockard's observations may now be considered as no longer existing. For the 'independent' lenses recorded by these authors in teratophthalmic embryos are due solely to 'infection' of the ectoderm with particles of blastolyzed optic cup

substance. In a similar manner we may perhaps yet succeed in obtaining identical results in those amphibian species which at present form the vexing exception to the rule of the dependent differentiation of the lens. The chemical method has some advantages over the most refined mechanical method of experimentation. Might it not perhaps, if employed on amphibian eggs, help to harmonize the contradicting results of the experiments with mechanical methods? Judging from results of some preliminary experiments[6] which I have performed with acetone solutions on frog eggs, I am inclined to think that blastolytic teratophthalmia can in these eggs also be produced. And it seems safe to expect that free lenses which so often occur in teratophthalmia, may also be found in such teratophthalmic amphibian embryos. I venture to predict that results similar to those in the embryo here described may be obtained, if weak solutions of acetone and long exposures be employed.

Another possibility for the origin of free lenses in teratophthalmic embryos is suggested by the retinal lentoid of our embryo (fig. 5). I regard the latter as a tissue heteromorphosis due to a local chemical injury of the potential retina. This injury may not be strong enough to destroy the part affected, but rather it may consist in so decreasing by chemical alteration its chemomorphic potentiality as to cause its ultimate differentiation into a structure less complex than it was 'destined' to form. This embryonic heteromorphosis has its analogue in the regenerative heteromorphosis presented by Fischel's ('02) retinal lentoids, the differentiation of which resulted from mechanical injury of parts of the retina. It would seem not improbable that in Fischel's experiments, too, chemical alteration which resulted from the mechanical destruction has so decreased the chemomorphic capability of the injured parts of the retina that the potency for hypotypical regeneration (lentoids) only has remained. However this may be, it is evident that optic cup substance may under certain circumstances develop into bodies of lens-like structure. If we now recall the fact that eye terata result from blastolytic injury of the potential optic cup, it is easy

[6] Not published.

to imagine that some free lentoids found in such embryos may owe their origin to heteromorphosis from dispersed optic cup fragments. However, it seems doubtful whether fully differentiated free lenses of large size originate from this source.

While, of course, this possibility cannot be entirely disregarded, the evidence points to the conclusion that the fully differentiated free lenses found in some teratophthalmic embryos are due to a chemical stimulus of blastolyzed potential optic cup substance on any part of the ectoderm with which it may chance to come into contact. It is quite possible that this stimulus (the lentogenic reaction) is in the nature of a catalytic reaction such as was assumed by Herbst ('01) to underlie the differentiation of the secondary sexual characters owing to products of internal secretion of the sex glands.

Reactions of this nature (autocatalysis) have in recent years been assumed by J. Loeb ('02, '09), Robertson ('08) and Hagedoorn ('11) and others to underlie the mechanisms of development, growth and inheritance. According to Hagedoorn the hereditary (genetic) factors are to be regarded as autocatalytic substances. A similar view has been advanced recently also by Goldschmidt ('16) who pointed out a quantitative analogy between genetic factors and enzyme reactions in the inheritance of wing pigmentation by various moth hybrids of a known gametic constitution.

The significance of these views is obvious. For, if their correctness should be established, we should gain a very deep insight into the governing forces of development. The evidence will be the more valuable if it is furnished by both genetics and experimental embryology. One instance of such evidence will, I think, eventually be found to be presented by the mode of origin of the lens in vertebrates.

MARCH 29, 1916

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PLATE 1

EXPLANATION OF FIGURES

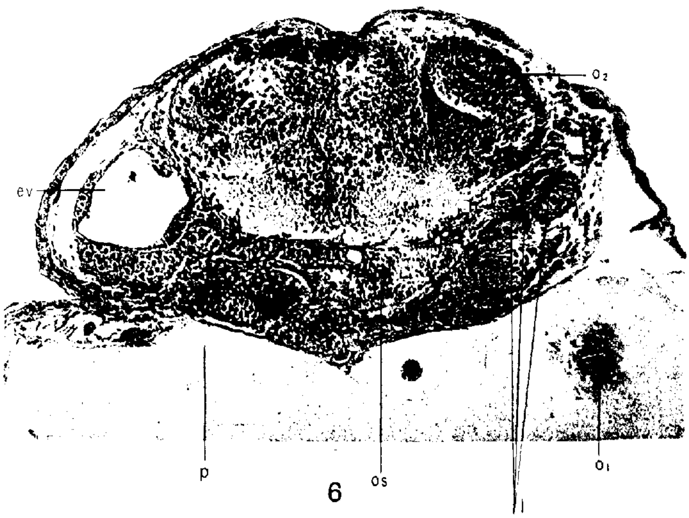
3 to 5 Photomicrographs of transverse sections through the head of the embryo in figure 1. *b.*, brain; *l.*, lentoids; *m.*, mouth cavity; *L.*, lens; *p.*, plasma in oedematous pericardium; *r.l.*, retinal lentoid; *o.s.*, dissociated optic cup substance. × 150.


b
m
l
l
3
b
L
p
l
4
rl
p
os
5

PLATE 2

EXPLANATION OF FIGURES

6 and 7 Photomicrographs of sections at more posterior levels of the same embryo. *o.s.*, dissociated optic cup substance in the oral cavity; o_1, the last trace of the right eye; o_2, the second optic cup of the right side; *l.*, lentoids; *p.*, plasma in pericardium; *e.v.*, ear-vesicle. × 150.



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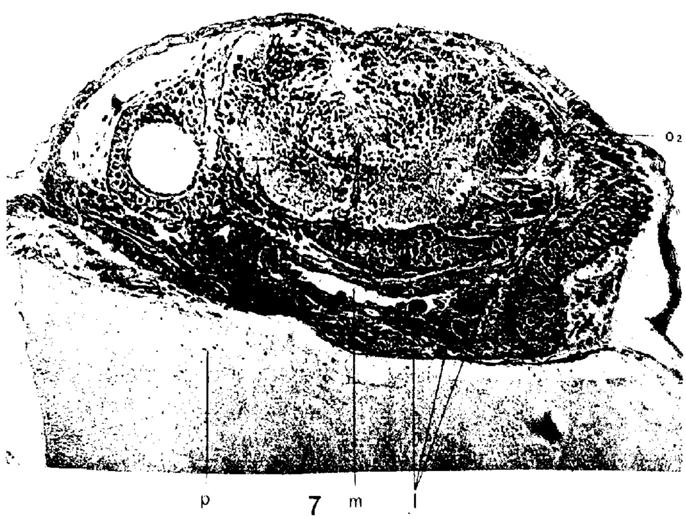

7

THE PHYSIOLOGY OF CELL-DIVISION

VI. RHYTHMICAL CHANGES IN THE RESISTANCE OF THE DIVIDING SEA-URCHIN EGG TO HYPOTONIC SEA WATER AND THEIR PHYSIOLOGICAL SIGNIFICANCE

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INTRODUCTION

In experiments performed at Woods Hole during the summer of 1901 Lyon[1] found that dividing Arbacia eggs varied greatly in their susceptibility to cyanide poisoning at different periods of the cell-division cycle. Eggs placed in cyanide-containing sea-water (m/100 to m/200 KCN) some time previously to the first cleavage, but not too soon (later than 15 or 20 minutes) after fertilization, withstood exposures of several hours without losing the power of development; while eggs exposed to the same solution at the time of cytoplasmic division were promptly killed. After the completion of cleavage a return of resistance was observed; this was followed by a second decline at the time of the second cleavage. It has been shown by Loeb[2] that the resistance to cyanide poisoning is much greater in the unfertilized than in the fertilized egg; Lyon found that fertilization is immediately succeeded by a period of high susceptibility, lasting some ten or fifteen minutes; then follows a resistant period which is terminated by the first cleavage. At first it was uncertain whether the resistance reached its minimum during or immediately after cleavage; Lyon inferred the latter, and supposed that the cyanide acted by preventing the oxidations necessary for the nuclear resynthesis following cytoplasmic division. A later examination of the question by Mathews[3]

[1] E. P. Lyon, Amer. Journ. Physiol., 1902, vol. 7, p. 56.
[2] J. Loeb, Biochem. Zeitschr. 1906 vol. 1, p. 200.
[3] A. P. Mathews, Biol. Bull., 1906, vol. 11, p. 137.

indicated, however, that the period of maximum susceptibility is "immediately before and during segmentation," and that just after segmentation the egg becomes relatively highly resistant. This conclusion was supported by observations of Spaulding[4] on the variations of resistance to weak solutions of ether ($\frac{1}{64}$ per cent in sea-water), which gave an unusually clear result; the resistance proved high (with the exception of the period immediately following fertilization) "up to either just before or the beginning of the first cleavage; during the early part of cleavage it falls to zero, with a sharp rise afterwards and a fall at the second segmentation" (p. 232). Spaulding also found a similar though less definitely marked variation of susceptibility to acid and salt-solutions (pure isotonic KCl and NaCl). A rhythmical variation in the physiological state of the cell is thus associated with the rhythm of the cell-division process. This variation involves changes in both the metabolism and the physical condition. Lyon[5] showed that the eggs were most readily injured by heat (e.g., 35° for 5 minutes) at the time of division, and recovered resistance after cleavage was complete; he also made the important observation that the evolution of CO_2 follows a parallel course, reaching a maximum at the time of cleavage. This indicates a rhythm of oxidation-processes; and the resistance to lack of oxygen (hydrogen atmosphere) appears to follow a similar rhythm. More recently Conklin[6] has described results of a related kind in experiments on Crepidula eggs; various abnormal conditions (warm sea-water, dilute and concentrated sea-water, ether, strong electrical currents) produce abnormalities (of cleavage, distribution of chromosomes, etc.) in these eggs; "the greatest changes are produced when the eggs are in some phase of kinesis at the beginning of the experiments, while stages of interkinesis are affected relatively little;" in general, "eggs are much more susceptible to injury during division than during rest."[7] Similarly A. R. Moore

[4] E. G. Spaulding, Biol. Bull., 1904, vol. 6, p. 224.

[5] Lyon, Amer. Journ. Physiol., 1904, vol. 11, p. 52.

[6] E. G. Conklin, Journ. Acad. Nat. Sc. Philadelphia, 1912, vol. 15, 2nd ser., p. 503.

[7] Loc. cit., pp. 525, 538.

finds that the resistance of sea-urchin eggs to injury by hypertonic sea-water is least "immediately before and during each cytoplasmic division, and that the maximal resistance is shown 35 to 45 minutes after fertilization and just after each division."[8]

It thus appears that the resistance to a variety of injurious agencies is least at the time of cytoplasmic division, i.e., while the form of the cell is undergoing rapid change. This change of form indicates alteration of surface-tension, which again suggests alteration of electrical surface-polarization, an effect which would result from an increased permeability of the electrically polarized plasma-membrane to electrolytes.[9] Hence it is possible to refer these several effects primarily to alterations of the surface layer of the egg. The analogy between cell-division, an essentially rhythmical process, and the phenomena of rhythmical auto-stimulation in heart-muscle cells, cilia, or respiratory nerve cells, favors such an interpretation, since there is ample evidence that stimulation is associated with a temporary increase of surface-permeability. Accordingly I have put forward the hypothesis that these rhythmical variations in the physiological state of the egg, during the cycle of cell division, are essentially the result of variations in the physical condition, especially the permeability, of the surface-film or plasma-membrane, the latter undergoing a reversible increase in permeability at the time of cleavage.[10] "A rhythm of alternate increase and decrease of permeability thus accompanies the rhythm of the mitotic process."[11] Any temporary loss of semi-permeability would be favorable to the entrance of poisons;[12] it would also decrease the electrical surface-polarization, and this change,

[8] A. R. Moore, Biol. Bull., 1915, vol. 28, p. 253; cf. p. 257.

[9] Cf. my paper in Biol. Bull. 1909, vol. 17, p. 204.

[10] Cf. R. S. Lillie, Biol. Bull., 1909, vol. 17, p. 188, cf. p. 207; Amer. Journ. physiol., 1910, vol. 26, p. 126, and 1911, vol 27, p. 289; Jour. Morph., 1911, vol. 22, p. 711.

[11] Cf. Amer. Journ. Physiol., 1910, vol. 26, p. 133.

[12] This might account for the greater toxicity of certain substances at this time, but not of all, since lipoid-soluble substances, e.g., ether, appear to penetrate the cell equally readily at all times. A general decrease of stability, or of resistance to abnormal conditions, (e.g., heat), seems to be associated with the increase of permeability.

judging from the analogy of the stimulation-process, would alter the metabolic processes, e.g., oxidations, within the cell; it would also involve increase of surface-tension, and under appropriate conditions a definite change of form would result.

Evidently this assumed increase of permeability and surface-tension cannot be uniform over the whole cell-surface;[13] in order to account for the definite and symmetrical change of form observed in cell-division, it seems necessary to assume that the surface-tension is chiefly increased over two symmetrically placed areas centering at the poles and extending to near the equator; these coincide with the regions of increased permeability and decreased electrical polarization. By the traction of these areas on the surface-protoplasm at the relatively unaltered equatorial zone, where surface-tension remains low, material is removed from this latter region, with the result that the cleavage-furrow is formed and progressively deepens. This hypothesis regards the egg as having the properties of a droplet of viscous fluid, and ascribes cytoplasmic division to surface-forces. The assumed distribution of the areas of altered surface-tension would account for the observed change of form;[14] the hypothesis

[13] This is evident, since all freely suspended homogeneous fluid droplets, whatever their surface-tension, have alike the spherical form. Departure from sphericity, under these conditions, means unequal tension at different portions of the surface. A symmetrical change of form, as in the dividing sea urchin egg, implies symmetrical distribution of the areas of altered surface-tension.

[14] Cf. the experiments and discussion of T. B. Robertson, Arch. f. Entwicklungsmech., 1909, vol. 27, p. 29, and 1913, vol 35, p. 692. Robertson's conclusions are rejected by McClendon (ibid., 1913, vol. 37, p. 233), on what seem to me insufficient grounds.

The considerations adduced by Donnan (Zeitschr. f. physik. Chem., 1899, vol. 31, p. 42), to explain why adjacent droplets in an emulsion of oil in weak soap solution do not fuse, also apply here. When two such drops accidentally come into contact, union is prevented because their approach increases the concentration of soap at the region of contact and there lowers the surface-tension; this causes a mutual withdrawal of the drops through the higher tension of the rest of the surface; i.e., the contiguous portions of the surfaces are drawn apart by the greater traction exerted by the non-contiguous portions. Similar reasoning will apply to a partly divided cell. See Donnan's diagram, p. 48 (also given in Freundlich's Kapillarchemie, p. 456). According to the present conception, the separation of the two blastomeres in the dividing egg is similarly due to the greater surface-tension of the circumpolar and 'temperate' zones of the cell-surface as compared with the equatorial zone.

also agrees with the facts about to be described, indicating that the properties of the plasma-membrane over the greater part of the cell-surface undergo profound alteration at the time when the cleavage-furrow is forming.[15]

Changes in the permeability of the plasma-membrane to diffusing substances imply changes in its general physical properties as well as in its osmotic behavior. In general the resistance which a gel (in which class the plasma membrane belongs) offers to diffusion, is a direct function of its density, i.e., colloid-content;[16] its mechanical properties (elasticity, tensile strength, etc.) are similarly determined. Hence the more permeable the plasma-membrane is to diffusion or to the passage of water, the less resistance should the egg offer to osmotic distention in dilute sea-water. The entrance of water into fertilized eggs under such conditions is in fact several times more rapid than into unfertilized eggs.[17] This difference of behavior is undoubtedly an expression of the general increase of permeability which results from fertilization. Correspondingly we should expect to find an analogous difference between the behavior of eggs placed in dilute sea-water during and previously to cleavage, if the properties of the membrane do in fact undergo change at this time.

In the experiments about to be described I have investigated the behavior of fertilized Arbacia eggs in dilute sea-water at different periods of the cell-division cycle. A striking change in the properties of the plasma-membrane at the time of cleavage is in fact readily demonstrable. As soon as the cleavage-furrow begins to form, or a little earlier, the membrane shows a marked decrease in its extensibility, and the eggs undergo rapid cytolysis in dilute sea-water to which previously they were resistant. This unstable condition persists during the formation of the furrow; afters its completion its original resistance returns. The following section describes these phenomena in detail.

[15] Other factors probably enter in the change of surface-tension; see the general discussion below, p. 394.

[16] Cf. Freundlich, Kapillarchemie, p. 515; Bechhold. Die Kolloide in Biologie und Medizin, 1912, p. 48; Bechhold und Ziegler, Zeitschr. f. physik. Chem., 1906, vol. 56, p. 105; Ruhland, Biochem. Zeitschr., 1913, vol. 54, p. 59.

[17] R. S. Lillie, Amer. Journ. Physiol., 1916, vol. 40, p. 249.

EXPERIMENTAL

When placed in dilute sea-water Arbacia eggs take up water osmotically and swell; the rate of this change varies with the dilution of the medium and with the condition of the eggs, and is surprisingly slow in unfertilized eggs.[18] If the dilution is sufficient the eggs eventually undergo a disruption or cytolysis, due to the destruction of the normal semi-permeable properties of the plasma-membrane. The pigment then leaves the eggs and colors the water; the cell-contents turn a characteristic pink, and the protoplasm assumes a granular appearance. This form of cytolysis has definite and unmistakable characteristics.

Osmotic distention in dilute sea water is destructive to the egg only if it exceeds a critical limit, which appears to be determined by the properties of the plasma-membrane. The latter may undergo a certain increase in area without losing the normal semi-permeability; such a partly swollen egg on return to normal sea-water loses the excess of water, and if previously fertilized continues development;[19] or if unfertilized it may be fertilized and will then develop. A considerable addition to the normal water-content of the protoplasm may thus be made without permanent injury; this possible addition is greater in the case of the fertilized than of the unfertilized egg. Analogous conditions are found in other cells; the variations in the resistance of homologous cells from different animals are especially interesting in this relation, since some evidence exists that these variations are correlated with definite differences of lipoid-content. According to Höber the red corpuscles of the horse first begin to lose hæmoglobin in a NaCl solution of 0.68 per cent concentration; for the ox the corresponding limiting concentration is 0.58 per cent, for man 0.45 per cent.[20] Obviously these differences are not due to differences in the osmotic pressure of the cell-contents, since the plasma has the same freezing point in all cases. For some special reason the corpuscles require greater osmotic distention in some animals

[18] Cf. my paper just cited, p. 256.

[19] See table 5, p. 384.

[20] Physikalische Chemie der Zelle und der Gewebe, 4th Edition, 1914, p. 77.

than in others before the plasma-membrane loses its normal insulating and semi-permeable properties. This specific degree of resistance is correlated in a remarkable manner with the resistance to the cytolytic action of chemical agents like saponin. Rywosch found, using a series of corpuscles from different mammals, that there was an inverse relation between the resistance to hypotonic NaCl solution and the resistance to saponin, i.e., those corpuscles most resistant to hypotony were least resistant to saponin.[21] Since saponin appears to act by combining with the cell lipoids, lecithin and cholesterol,[22] it would seem that these differences depend on differences in the lipoid-content of the plasma-membrane. The analyses of Abderhalden and of Mayer and Schaeffer [23] indicate that the corpuscles least resistant to hypotony are those richest in cholesterol. The preponderance of cholesterol over lecithin in corpuscles, as well as in the artificial lipoid membranes of Pascucci,[24] seems to impart a resistance to the disintegrative action of saponin, at the same time rendering the cell less capable of resisting osmotic distention. Variations in the lipoid-content of the egg at different periods may thus form the basis of the above variations in the resistance to osmotic disruption. As indicating a possible relationship of this kind, I have recently called attention[25] to the interesting observation of Lyon and Schackell that the unfertilized Arbacia egg has a decidedly higher iodine-combining power than the fertilized egg;[26] this suggests that at fertilization there is a decrease in the iodine-combining constituents of the plasma-membrane, e.g., cholesterol or other unsaturated lipoids. It seems probable that similar variations may occur during the

[21] Rywosch, Arch. f. d. ges. Physiol., 1907, vol. 116, p. 229.

[22] Ransom, Deutsche med. Wochenschr., 1901, no. 13; Pascucci, Beiträge zur chem. Physiol. u. Path., 1905, vol 6, p. 552; Hausmann, ibid., p. 567; K. Meyer, ibid., 1908, vol. 11, p. 357; Windaus, Ber. d. deutsch. chem. Ges., 1909, vol. 42, p. 238.

[23] Abderhalden, Zeitschr. f. physiol. Chem., 1898, vol. 25, p. 65; Mayer and Schaeffer, Comptes rendus, 1912, vol. 155, p. 728. See also Kauders, Biochem. Zeitschr., 1913, vol. 55, p. 96.

[24] Loc. cit.

[25] Amer. Journ. Physiol., 1916, vol. 40, p. 265.

[26] Lyon and Shackell, Science, N.S., 1910, vol. 32, p. 249.

cell-division cycle, but evidently analytical and other data are required to decide this question. The resistance to saponin at different stages has not yet been determined; but Spaulding's observations[27] show that the resistance to ether declines markedly at the time of cytoplasmic division.

The resistance of the eggs to osmotic disruption in dilute sea-water thus forms a convenient index of the physical condition of the plasma-membrane. As already mentioned, this resistance is lower in the unfertilized than in the fertilized egg (except at the time of cytoplasmic cleavage); i.e., in the unfertilized egg cytolysis occurs at a lower degree of osmotic distention than in the fertilized egg. A further and probably related difference, also mentioned above, is that water enters the unfertilized egg much more slowly than the fertilized egg; i.e., high resistance to entrance of water—or a relatively waterproof character—appears to involve a relatively slight extensibility of the membrane.

The degree of dilution required to cytolyze the majority of unfertilized Arbacia eggs in half an hour is about 60 volumes per cent (60 volumes tap water plus 40 sea-water). At 55 per cent dilution most eggs remained intact after 4 hours; at 57.5 per cent about half of the eggs were cytolyzed after $1\frac{1}{2}$ hours and all after 4 hours. Fertilized eggs withstand exposure to all of these dilutions for several hours without cytolysis.

Table 1 gives the results of experiments with different dilutions of sea-water. The experiments were performed on different days, each with a separate lot of eggs. In each experiment fertilized and unfertilized eggs from the same lot were placed in the dilute sea-water—the fertilized eggs about 12 minutes after fertilization—and the proportion of cytolyzed eggs was estimated after different intervals. The approximate proportion of eggs found cytolyzed after 20 minutes in the dilute sea-water is given in the table (see table 1).

The greater resistance of the fertilized eggs to osmotic distension is evident from the table. This high resistance remains until shortly before the appearance of the cleavage-furrow; it

[27] Loc. cit.

TABLE 1

	DILUTION (VOLS. PER CENT TAP WATER)	PROPORTION OF EGGS FOUND CYTOLYZED AFTER 20 TO 25 MINUTES IN DILUTE SEA-WATER	
		Unfertilized	Fertilized
1	52.5	ca. 2 to 3 per cent	0
2	55	ca. 1 per cent (at 20 m.)	0
3	57.5	20 to 25 per cent	0
4	60	ca. 50 per cent (av. of 5 exps.)	0
5	62.5	95 per cent or more	Almost none
6	65	All	A few (5 to 10 per cent)

After three hours in sea-water of 62.5 per cent dilution the majority of fertilized eggs were still uncytolyzed, though greatly distended; at 65 per cent about half were cytolyzed and depigmented, the remainder swollen but intact.

then rapidly declines, and at the time of appearance of the furrow the membrane is only slightly extensible; at this time many eggs undergo cytolysis in dilutions so low as 40 or 42.5 per cent, and in a dilution of 60 per cent all are rapidly and completely cytolyzed, the majority within two or three minutes. This state of low resistance persists during the three or four minutes occupied by the formation and extension of the cleavage-furrow; after the latter is complete the former resistance quickly returns. At ten minutes after cleavage the resistance is found to be practically the same as at ten minutes before cleavage. Thus during the period of fifteen or twenty minutes occupied by the processes, preparatory and active, of cytoplasmic division, the membrane undergoes a reversible change of state which profoundly affects its extensibility and coherence, and presumably its permeability and other general properties. A similar change is found at the second and third cleavage, and probably occurs at all cell-divisions (see table 2).

In the following table the results of a typical experiment are described in detail.

It is clear from these results that the eggs very readily undergo cytolysis at the time when the cell-body is dividing, and become again resistant in the intervals between divisions. These observations were not carried beyond the third cleavage, because of increasing variation in the state of different eggs, and

the beginning of divergences in the cleavage-rates of the blastomeres. For example, of eggs exposed at the time of the third cleavage a considerable proportion (ca. 15 per cent) remained uncytolyzed after fifteen minutes in the dilute sea-water; presumably these represent the more rapidly cleaving and the more slowly cleaving fractions, which had either partly regained

TABLE 2

August 17, 1915. Arbacia eggs were fertilized at 11.05 a.m. From this fertilized lot portions were transferred to dilute sea-water at the following times: (1) shortly after fertilization (11.07 and 11.11), (2) midway between fertilization and cleavage (11.29), (3) at a time when the cleavage furrow was first visible in a majority of eggs (11.50), (4) and (5) after the completion of cleavage (12.00 and 12.08), (6) at the beginning of the second cleavage (12.20), (7) ten minutes after the completion of the second cleavage (12.33), (8) at the beginning of the third cleavage (12.48), and (9) ten minutes after the completion of the third cleavage (1.00). Two dilutions of sea-water were used: (a) 60 volumes tap water plus 40 sea-water, and (b) 65 tap water plus 35 sea-water. The second column gives the condition of the eggs at three minutes and at fifteen minutes after placing in the 60 per cent dilute sea-water

	TIME OF PLACING IN DILUTE SEA-WATER AND CONDITION OF EGGS AT THAT TIME	EFFECT OF 60 PER CENT DILUTE SEA-WATER ON EGGS (CONDITION AFTER 3 AND 15 MINUTES)
1	11.07 and 11.11 (eggs with fertilization membranes; otherwise unchanged).	Eggs swollen, but intact.
2	11.29 (unchanged).	Eggs swollen, but intact.
3	11.50 (cleavage-furrow visible in most eggs).	3 m.: ca. 20 cytolyzed; 15 m.: nearly all cytolyzed (ca. 90 per cent).
4	12.00 (all in 2-cell stage).	3 m.: practically all intact; 15 m.: great majority intact; ca. 10 per cent cytolyzed.
5	12.08 (all in 2-cell stage).	3 m.: practically all intact; 15 m. 95 per cent or more intact; a few cytolyzed.
6	12.20 (about one-third in early 4-cell).	3 m.: ca. 50 per cent cytolyzed. 15 m.: ca. 90 per cent cytolyzed.
7	12.33 (all in completed 4-cell stage	3 m.: practically all intact; 15 m.: great majority (ca. 80 per cent) intact; remainder cytolyzed.
8	12.48 (about one-third in early 8-cell).	3 m.: ca. 30 to 40 per cent cytolyzed: 15 m.: 80 to 90 per cent cytolyzed: a fair proportion remain intact.
9	1.00 (all in completed 8-cell stage).	3 m.: nearly all intact; 15 m.: great majority intact (ca. 75 to 80 per cent); remainder cytolyzed.

resistance or had not wholly lost it; the great majority, however, underwent prompt and complete cytolysis. In the series with 65 per cent dilution the results were essentially the same, the only difference being that cytolysis was more rapid, and a larger proportion of eggs exposed in the intervals between cleavages underwent destruction. This is to be expected, since both decline and recovery of resistance are continuous, the former (e.g.) beginning before there is any external evidence of cleavage. Hence at a stage when the eggs are still resistant to the 60 per cent dilution they are destroyed by the 65 per cent. A repetition of the above two series gave the same result. In four other series similar observations were made for the first two cleavages, but not for the third.

The change in the susceptibility of the membrane begins several minutes before any external indication of cleavage can be seen. This shows that the change is progressive, reaching a climax at the time when the cleavage-furrow begins to form. In order to trace its course in more detail, experiments were performed in which eggs were transferred from normal to dilute sea-water at regular intervals of two minutes during a total period of about twenty-four minutes, beginning ten minutes before and ending ten minutes after the period of visible change of form. Eggs were also exposed at two-minute intervals during the first eight minutes after fertilization. Table 3 gives the description of a typical experiment of this kind. The procedure was the same as before; sea-water of 60 per cent dilution was used. The first column gives the interval after fertilization at which the eggs were placed in the dilute sea-water, also the condition of the eggs at that time; the second column gives the approximate proportion of eggs found cytolyzed after exposures lasting respectively three and thirty minutes (see table 3).

These observations show that the eggs resist the disintegrative action of the osmotic swelling until shortly before the first appearance of the cleavage-furrow; they also show that the properties of the membrane begin to change several minutes before there is any visible change in the form of the egg. In the above series the first furrow is seen at forty-eight minutes

TABLE 3

Experiment of August 19. Eggs were fertilized at 11.04 a.m. and placed in dilute sea water (60 per cent dilution) at the following intervals after fertilization

	TIME OF PLACING IN DILUTE SEA-WATER AND CONDITION OF EGGS AT THAT TIME	EFFECT OF DILUTE SEA-WATER ON EGGS (CONDITION AFTER 3 AND 30 MINUTES)
1	2 m. (all with fertilization membranes).	3 m.: all eggs swollen but intact, many extra-ovates. 30 m.: all eggs intact; 30 to 40 per cent extra-ovates.
2	4 m. (unchanged).	3 m. and 30 m.: all eggs intact, but few extra-ovates, ca. 1 per cent.
3	6 m. (unchanged).	3 m. and 30 m.: all intact; almost no extra-ovates.
4	8 m. (unchanged).	3 m. and 30 m.: all intact.
5	40 m. (eggs unchanged).	3 m.: all intact. 30 m.: nearly all intact; 1 to 2 per cent cytolyzed.
6	42 m. (unchanged).	3 m.: all intact. 30 m.: nearly all intact; ca. 4 to 5 per cent cytolyzed.
7	44 m. (unchanged).	3 m.: nearly all intact; a few cytolyzed. 30 m.: ca. 10 per cent cytolyzed; the rest intact.
8	46 m. (still round and unchanged).	3 m.: ca. 20 per cent cytolyzed; the rest intact. 30 m.: ca. 35 to 45 per cent cytolyzed.
9	48 m. (cleavage-furrow beginning in a few eggs).	3 m.: ca. 50 per cent cytolyzed. 30 m.: great majority cytolyzed (ca. 80 to 85 per cent).
10	50 m. (furrow visible in about half the eggs)	3 m.: ca. 65 to 70 per cent cytolyzed. 30 m.: 90 per cent or more cytolyzed.
11	52 m. (most eggs cleaving).	3 m.: great majority cytolyzed. 30 m.: almost all cytolyzed (ca. 95 per cent).
12	54 m. (90 per cent or more in 2-cell stage).	3 m.: ca. 35 to 45 per cent cytolyzed. 30 m.: now many intact eggs; 60 to 70 per cent cytolyzed.
13	56 m. (all in 2-cell stage).	3 m.: two-thirds or more intact. 30 m.: most eggs intact; 25 to 35 per cent cytolyzed.
14	58 m. (all in 2-cell stage).	3 m.: great majority intact. 30 m.: great majority intact; ca. 20 to 25 per cent cytolyzed.
15	60 m.	3 m.: ca. 90 per cent intact. 30 m.: great majority intact; ca. 10 to 15 per cent cytolyzed.
16	62 m.	3 m.: nearly all intact. 30 m.: nearly all intact; ca. 5 per cent cytolyzed.

after fertilization, but at forty minutes there is already a slight and at forty-two minutes a distinct increase in susceptibility; this rapidly increases to a maximum which coincides with the time at which the furrow is forming in the great majority of eggs. When the furrow is complete the former resistance rapidly returns. Ten minutes after cleavage the condition of the membrane is apparently nearly if not quite the same as ten minutes before the first appearance of the furrow. A few susceptible eggs are usually found at this time; however, and it is possible that after cleavage has once started the interval between cleavages is too short to admit of complete recovery of the condition characteristic of the uncleaved egg.

In the series of table 3 the maximum of susceptibility was found at 52 minutes after fertilization, at a time when the majority of eggs were undergoing change of form. By using less dilute sea-water the precise time of this maximum can be determined more accurately, since with sufficiently low dilutions (e.g., 40 to 45 per cent) only those eggs are cytolyzed which have reached the most susceptible stage. Conversely, by using sea-water more dilute than 60 per cent, the preliminary increase of susceptibility can be detected considerably earlier than ten minutes before the first appearance of the furrow. By thus varying the dilution of the medium the progressive character of the change in the plasma-membrane can be demonstrated with great clearness.

Table 4 gives a summary of all of last summer's experiments which were carried out in a manner similar to the above. The dilutions range from 52.5 to 62.5 volumes per cent. The table gives the times after fertilization at which eggs were placed in the dilute sea-water, and the approximate proportion of eggs found cytolyzed after exposures of respectively three and thirty minutes. In all cases a well defined maximum of susceptibility was found at the time when the cleavage-furrow was in process of formation in most eggs. The formation and extension of the furrow occupy three or four minutes at this temperature (21 to 23°) (see table 4).

TABLE 4

DILUTION OF SEA-WATER		TIME OF PLACING IN DILUTE SEA-WATER (MINUTES AFTER FERTILIZATION) AND APPROXIMATE PERCENTAGE OF EGGS CYTOLYZED AFTER 3 AND 30 MINUTES EXPOSURE													
		2-10 m.	38 m.	40 m.	42 m.	44 m.	46 m.	48 m.	50 m.	52 m.	54 m.	56 m.	58 m.	60 m.	62 m.
Aug. 18. 60 volume per cent...	3 m.	0													
	30 m.	0						25–35	>50	60–65	>90	ca. 75–80	20–25	10–15	ca. 5
Aug. 19. 60 volume per cent...	3 m.	0													
	30 m.	0		ca. 1–2	ca. 4–5	ca. 10	35–45	80–85	90+	ca. 95	70–80	25–35	20–25	10–15	ca. 5
Aug. 20. 60 volume per cent...	3 m.	0		0	1–2	ca. 5	10–15	30–40	>50	30–40	5–10	ca. 5	ca. 5	<5	ca. 1
	30 m.	0		ca. 5	5–10	30–40	50–60	60–70	70–80	60–70	20–30	15–20	10–15	ca. 10	ca. 2–3
Aug. 21. 62.5 volume per cent...	3 m.	0	0	ca. 0	ca. 0	5–10	20–30	ca. 50	60–70	70–80	35–45	25–35	10–15	ca. 5	ca. 5
	30 m.	0	10–20	25–35	35–45	60–70	80–90	>90	ca. 95	75–85	55–65	ca. 50	25–35	10–15	10–15
Aug 23. 55 volume per cent...	3 m.	0	0	0	0	2–3	ca 2–4	ca. 10	10–15	<5	<1	<1	ca. 0	ca. 0	ca. 0
	30 m.	0	0	0	ca. 5	25–35	40–50	60–70	55–65	30–40	ca. 10	<5	1–2	<1	<1
Aug. 24. 57.5 volume per cent...	3 m.	0	0	0	0	ca. 1	ca. 10	20–30	25–35	10–15	<5	ca. 1	<1	ca. 0	ca. 0
	30 m.	0	1–2	2–3	ca. 5	ca. 50	80–90	ca. 90	ca. 90	ca. 50	25–35	ca. 5	ca. 1–2	ca. 1	<1
Aug. 25. 52.5 volume per cent...	3 m.	0	0	0	0	<1	<5	ca. 5	ca. 5	ca. 5	<1	<1	ca. 0	ca. 0	ca. 0
	30 m.	0	0	0	ca. 5	10–15	30–40	40–50	50–55	30–40	15–20	5–10	4–5	ca. 1	ca. 0

In the experiments cited in table 4 the period of maximum susceptibility varies between 48 and 54 minutes after fertilization; in each series there is a period corresponding to the change of form and lasting about four minutes, during which the susceptibility remains about the same; the resistance then returns rapidly; its return evidently signifies the completion of the cleavage-process. With the most dilute sea-water used (62.5 per cent) a well marked increase of susceptibility is apparent twelve minutes before the period of maximum susceptibility. In most series a few eggs remain susceptible ten minutes after cleavage is complete in the great majority; these probably represent chiefly the minority of slowly cleaving eggs; possibly, however, as already suggested, the recovered resistance is not quite equal to that of the uncleaved egg.

Another and independent method of demonstrating these variations of resistance is by returning eggs to normal sea-water after exposure to the dilute medium for a definite period, and later determining the proportion which continue development to the blastula stage. In several series of such experiments the surviving eggs were always found much more numerous in those lots which had been exposed before cleavage or in the intervals between cleavages; while few if any eggs exposed during the formation of the furrow continued development. The five per cent or fewer eggs which formed blastulæ in Experiments 6 to 9 B (table 5) undoubtedly represent the relatively resistant minority which had not yet begun to cleave, or had completed cleavage, at the time of placing in the dilute sea-water.

The degree to which the resistance changes at the time of cytoplasmic cleavage is perhaps best shown by comparing the effects of a graded series of dilutions upon the same lot of eggs, part of these being placed in the dilute sea-water well before the beginning of cleavage (about midway between fertilization and the first cleavage), and part at the time when the furrow is forming in most eggs. Such an experiment shows that at the height of susceptibility many eggs are destroyed by dilutions so low as 40 volumes per cent; while in order to cause an equally rapid cytolysis in the uncleaved eggs, dilutions of 65 to 70 per cent are

TABLE 5

August 28. Eggs were fertilized at 11.20. After sixteen minutes in normal sea-water, part (A) were transferred to a series of dishes containing dilute sea-water of the compositions given in the first column; twenty-five minutes later (12.01), at a time when cleavage was beginning in most eggs, a second part (B) were similarly transferred. The condition of the eggs in the dilute sea water was observed at intervals. From each dish some eggs were returned to normal sea-water after an exposure of fifteen minutes; the proportion of these eggs continuing development was determined next day. The table gives for each dilution the condition of the eggs as observed after twenty minutes exposure; also the approximate proportion of eggs that formed blastulæ on return to sea water after fifteen minutes exposure

	Composition of dilute sea-water (vols. per cent tap water)	A. Eggs placed im dilute sea-water before cleavage		B. Eggs placed in dilute sea-water during cleavage	
		Condition of eggs after 20 minutes in dilute sea-water	Development after exposure of 15 minutes to dilute sea-water	Condition of eggs after 20 minutes in dilute sea-water	Development after exposure of 15 minutes to dilute sea-water
1	40			Most are intact; ca. 10 to 15 per cent cytolyzed.	Majority form blastulæ.
2	42.5			ca. 20 to 25 per cent cytolyzed; remainder intact.	A minority (30 to 40 per cent) form blastulæ; the rest dead.
3	45	Swollen but all intact.	Nearly all form blastulæ.	ca. 25 to 30 per cent cytolyzed; the rest intact.	Most dead next day; 25 to 35 per cent form blastulæ.
4	47.5	All intact.	Nearly all form blastulæ.	35 to 45 per cent cytolyzed; the rest intact.	ca. 25 to 30 per cent form blastulæ.
5	50	All intact.	Great majority form blastulæ.	40 to 50 per cent cytolyzed.	ca. 15 to 20 per cent form blastulæ.

6	52.5	All intact, swollen.	Most form blastulæ.	More than 50 per cent cytolyzed.	Few blastulæ next day; ca. 5 per cent; the rest dead.
7	55	All intact, swollen.	ca. 50 per cent form blastulæ.	60 to 70 per cent cytolyzed.	ca. 5 per cent form blastulæ.
8	57.5	All intact, swollen.	Less than half form blastulæ.	ca. 90 per cent cytolyzed.	A few (ca. 5 per cent) blastulæ; the rest dead.
9	60	Almost all intact.	Most eggs die; 20 to 30 per cent form blastulæ.	95 per cent or more cytolyzed.	Nearly all dead; ca. 2 to 3 per cent blastulæ.
10	62.5	Great majority intact; ca. 5 to 10 per cent cytolyzed.	Few eggs form blastulæ; ca. 5 per cent.	All cytolyzed.	None develop.
11	65	40 to 50 per cent cytolyzed; the rest swollen but intact.	Nearly all dead next day; a few blastulæ (<1 per cent).	All cytolyzed.	None develop.

TABLE 6

	Date and composition of dilute sea-water		Proportion of extra-ovates in eggs placed in dilute sea-water at different intervals after fertilization		
			2 m	4 m	6 m
			per cent	per cent	
1	August 17.	65 volume per cent	Many		Few
2	August 18.	60 volume per cent	10–15	1–2	Almost none
3	August 19.	60 volume per cent	30–40	ca. 1	Almost none
4	August 20.	60 volume per cent	20–30	ca. 1	Almost none
5	August 21.	60 volume per cent	20–30	<1	None
6	August 23.	55 volume per cent	ca. 10	ca. 1	Few (< 1 per cent)
7	August 24.	57.5 volume per cent	ca. 10–15	ca. 1	Few (< 1 per cent)
8	August 25.	52.5 volume per cent	ca. 10–15	ca. 1	Few (< 1 per cent)

sile strength undergo change during the first few minutes after separation from the egg-surface. This suggests properties like those of a secretion which hardens on contact with sea-water, such as the secretion forming the resistant chorion of Fundulus eggs. Most observations indicate, however, that the fertilization-membrane is really the separated surface-lamella of the unfertilized egg.[31] Previously to fertilization this apparently forms an intimate part of the outer protoplasmic layer or cortical zone; probably it then partakes in the metabolism of the latter and its semi-permeability is thus preserved.[32] When the egg responds to the membrane-forming agent, the surface lamella is separated from the cell-surface, possibly forced outward by the osmotic pressure or secretion-pressure of the surface-protoplasm; its properties then change, perhaps because of the cessation of metabolism. Heilbrunn also describes observations indicating physical changes in the membrane after separation; these changes are in the direction of an increase of permeability to salts; according to the above observations this change runs parallel with a loss of extensibility.[33]

[31] Cf. my paper in Amer. Journ. Physiol., 1911, vol. 27, p. 300; L. Heilbrunn, Biol. Bull., 1915, vol. 29, p. 158.

[32] The preservation of semi-permeability is a function of cell-metabolism; cf. my recent paper. loc. cit, 1916, p. 265.

[33] Compare Heilbrunn, loc. cit., p. 160. Heilbrunn infers an increased rigidity in the membrane, due to a coagulative and dehydrative change in its substance (p. 167).

GENERAL DISCUSSION

The foregoing experiments afford for the first time clear proof that a reversible change in the properties of the plasma-membrane is associated with the division of the cell-body during mitosis. This change is in the direction of greater instability and lower coherence, and apparently of greater permeability to diffusing water-soluble substances. According to the membrane-theory of the bioelectric processes, such a change should be accompanied by an electrical variation similar to that of stimulation; evidence that such variations exist is afforded by Miss Hyde's observations on dividing fish eggs.[34] There is, however, need of further observations in this field.

The close analogy between cell-division and other forms of response[35]—particularly the response of irritable elements to electrical or other stimulation, where a decrease of surface-polarization is undoubtedly the primary or critical event—gives to the above facts a definite bearing on the general theory of stimulation. They support the view that a reversible increase in the permeability of the plasma-membrane is an essential part of the stimulation-process; this change apparently determines the change of surface-polarization, and hence the metabolic and other effects within the irritable element.[36] According to this view, a reversible surface-change of a kind similar to that associated with cell-division, only relatively rapid, is a normal feature of excitation in irritable cells and elements.

The relations of such surface-changes to the entire cell-division process are far from clear in detail. Certain possibilities have already been indicated;[37] thus it is to be assumed that the plasma-membrane of the resting egg is the seat of an electrical demarcation-potential similar to that of other cells (muscle,

[34] I. H. Hyde, Amer. Journ. Physiol., 1904, vol. 12, p. 241.

[35] I have recently discussed this analogy in a paper in The Journal of Experimental Zoology, 1913, vol. 15, p. 23. Cf. also Amer. Journ. Physiol., 1910, vol. 26, p. 106.

[36] For evidence of the connection between changes of permeability and stimulation cf. Amer. Journ. Physiol., 1911, vol. 28, p. 197.

[37] Page 372 above.

etc.); in such a case an increase of permeability to electrolytes will involve decreased electrical polarization, and by the Lippmann-Helmholtz law of electrocapillarity, also increased surface-tension. With appropriate localization of the altered areas, and a sufficient increase of surface-tension, a definite change of form would result, as already indicated.

This, however, is only one possible result of the assumed change of surface-polarization. The complete effects of such a change upon the physico-chemical conditions within the dividing cell cannot be determined at present, and form a problem for future research. This problem will be simplified—in some of its terms at least—if it is recognized that dividing cells undergo, at the time of change of form, surface-changes similar to those which irritable elements like muscle-cells or nerve-fibers undergo at the time of stimulation, and of which the bioelectric variations are the most definite index. The time-relations of these changes are known to differ from cell to cell,[38] and the precise consequences within any particular cell vary according to the specific constitution of the latter. But in all cases the same general problem is met, namely: what are the nature and conditions of the effects, chemical and other, resulting within the cell from alterations of surface-polarization? Progress will have to be made in this fundamental problem before the relation of the membrane-changes to cell-division can be elucidated in detail.

Cytoplasmic radiations appear to play a highly important part in cell-division, although they vary greatly in their development in different cells, and are usually absent in amitosis. Their nature is still a subject of investigation; but the evidence[39] appears to favor the view that they are essentially the expression of local differences of electrical potential within the cell,—the polarized particles in the electrical field becoming oriented

[38] For a table giving the time-relations of the bioelectric variations in a large number of tissues and organisms, cf. Amer. Journ. Physiol., 1914, vol. 34, p. 417.

[39] Especially the regular and symmetrical curvature which they show in the spindle-area, and which seems inexplicable on any other hypothesis; mantle and other astral fibers are also often curved in a manner resembling the lines of force of intersecting electrical fields.

along the electrical lines of force, and apparently in many instances fusing to form strands. There are a number of possibilities in the formation of such potential-gradients; these I have partly discussed in previous papers,[40] but some further consideration seems desirable at present. The theory of diffusion-potentials implies that any well marked increase in the permeability of the plasma-membrane to any intracellular electrolyte distributed uniformly throughout the protoplasm and of which the cation diffuses more rapidly than the anion (e.g., an acid), will form within the cell a potential-gradient, corresponding to the diffusion-gradient, with the central region negative relatively to the peripheral region.[41] In other words, for a certain time the concentration of the electrolyte will be higher at the central region than at the cell-surface—where it is diffusing into the surrounding medium—and during this time a corresponding potential-gradient must exist. The precise characteristics of such a gradient will depend on the distances concerned, on the relative migration rates of anion and cation through the protoplasm, and on the concentrations. The latter two factors are unknown, so that it is difficult to judge of the adequacy of such a hypothesis; it seems, however, possible that the typical development of a bipolar system of cytoplasmic radiations at the time of division may be due largely if not entirely, to the appearance of two symmetrical areas of increased permeability centering at the poles and extending toward the equator, as suggested above—the electrical fields

[40] Biol. Bull., 1909, vol. 17, p. 188; Amer. Journ. Physiol., 1910, vol. 26, cf. p. 128, Jour. Morph., 1911, vol. 22, pp. 721 seq.

[41] I do not see why this simple hypothesis should be regarded as unintelligible (cf. Heilbrunn, Biological Bulletin, 1915, vol. 29, p. 184). It consists simply in a recognition that when a diffusion-preventing barrier is removed between two solutions, a diffusion-potential arises corresponding to the diffusion-gradient. Thus (e.g.) the P.D. between 10^{-x} normal and 10^{-x+1} normal HCl is approximately 0.05 volt; if two such solutions are separated by a semi-permeable membrane, and the permeability of the latter is then increased, evidently the acid at once begins to diffuse from the more to the less concentrated solution and continues until the concentration is uniform. As long as there is a diffusion-gradient there will be a corresponding potential-gradient. Similarly between the central and peripheral regions of the cytoplasm at the time of increased permeability.

formed by the assumed diffusion-fields having an orienting influence on the colloidal particles and determining their fusion along the radiating lines of force to form strands. The initial appearance of a general centrally directed cytoplasmic radiation in artificially activated sea-urchin eggs[42] suggests that a change of surface-permeability is adequate to produce this effect.

Other factors, however, undoubtedly enter in the production of cytoplasmic radiations, and the conditions are probably too complex for satisfactory analysis at present. The dividing cell, like irritable elements in general, no doubt reacts as a whole to changes in the electrical polarization of its surface; and certain localized metabolic changes (oxidations, etc.) presumably result, whose position and character depend on the nature, distribution, and physical conditions of the various substances present—i.e., on the pre-existing cell-organization. Cytological observation indicates that in dividing cells certain regions or foci of active chemical change (centrosomes) make their appearance, and become the centers of astral radiations; there is evidence that the material forming such centers persists in the interkinetic periods and becomes active only at certain times; these times, judging from the above observations, appear to be those when the cell-surface is undergoing the changes preparatory to division. It is possible that the change of surface-polarization (which is equivalent to stimulation) initiates oxidation-processes in each such area and leads to the formation of acid metabolic products; on account of the high migration rate of the hydrogen ion, each such area would then become negative relatively to adjoining regions; the radiations would thus be the expressions of localized potential-gradients resulting from the diffusion of electrolytes from chemically active regions;

[42] Cf. Wilson, Arch. f. Entwicklungsmechanik, 1901, vol. 12, p. 539; Hindle, ibid., 1911, vol. 31, p. 145; cf. p. 152.

Attention may also be drawn to the frequent arrangement of the cytoplasm in gland-cells in strands perpendicular to the free surface of the cell. It seems probable that this structure is due to essentially similar conditions, the outer or secreting surface of the plasma-membrane undergoing changes of permeability and polarization, with the production of diffusion-potentials between the superficial and the basal regions of the cell.

to each local diffusion-field would correspond an electrical field in which particles would be polarized and fuse as above suggested. I have pointed out in an earlier paper[43] that the positions adopted by chromosomes (which are undoubtedly negative particles[44]) relatively to astral centers indicates that the latter also are negatively charged; and there is little doubt that within the small distances concerned in cell-phenomena the relative positions of the different colloidal aggregates will be influenced by the charges which they carry;[45] in fact, many phenomena of normal and abnormal mitosis indicate that chromosomes are repelled from the centers of radiation. This is probably the cause of the characteristic arrangement relatively to these areas.[46] The similarity in the staining properties of chromosomes and astral centers also indicates that both carry negative charges. Each centrosomal area thus represents a negative area, i.e., the region of lowest potential in the local electrical field—corresponding to the diffusion-field—centering at each active centrosome. It is to be noted that if this conception is true, each hemisphere of the cell at the metaphase stage of mitosis is under

[43] Amer. Journ. Physiol., 1905, vol. 15, p. 46.

[44] This is obvious to the student of colloid chemistry. Nucleoproteins, on account of their acid properties, will be more negative, *i.e.*, will have higher isoelectric coefficients, than the majority of proteins. For example, according to Michaelis, the isoelectric coefficient of a nucleoprotein from the pancreas is 3×10^{-4}, as compared with values ranging from 2×10^{-5} to 2×10^{-7} for other proteins (cf. Höber, loc. cit., p. 330). The possibility of differentiating by means of acid and basic dyes the different proteins in cells (chromatin, etc.), after fixation with acid fixing fluids, depends in fact on this difference. The chromatin, being a nucleoprotein, remains negative, and hence stainable by basic dyes, after treatment with fluids which are sufficiently acid to render positive the charge on all the other protein colloidal particles present.

[45] Cytological evidence confirms this point of view. Cf. Conklin's general discussion of the mechanism of differentiation in The Anatomical Record. 1909, vol. 3, p. 153; "the movements of substances within cells take place largely through the instrumentality of the astral system of the mitotic figure, or of the entering spermatozoön," etc.

[46] See the experiments with magnetic models in my paper just cited (footnote 43). The chromosomes, previously to division, adopt positions of equilibrium midway between adjacent astral centers; this behavior is most evident in multipolar mitoses; for some especially striking examples cf. Kostanecki's paper on the parthenogenetic eggs of Mactra, Archiv für mikroskopische Anatomie, 1908, vol. 72, plates 14 and 15.

the influence of *two* conditions, each of which forms an electrical field negative centrally and positive peripherally: (1) that due directly to the increase of surface-permeability, and (2) that resulting from the diffusion of electrolytes from the chemically active centrosomal area. The total effect will be due to a summation of these two influences. According to such a conception, a response similar to that of irritable cells in general, resulting in definitely localized metabolic effects, is an essential factor in the mitotic process.

The above general conception of the physico-chemical nature of astral radiations is in agreement with many well known facts of experimental cytology. The fact that astral centers or centrosomes become active, i.e., develop radiations, concurrently with the changes of form of the cell-body, is especially noteworthy, and indicates a close connection between chemical activity in these regions and alterations in the surface-tension of the cell. Many indications point to the conclusion that this relation is at times one of effect, the primary or initiatory condition being the surface-change, as already suggested; at other times it is apparently one of cause, i.e., asters, once formed, may influence the surface-tension of the cell. The origination of supernumerary asters or cytasters in dividing eggs appears to illustrate the former condition; these structures, when present in the cytoplasm along with the division-asters, undergo parallel changes with the latter;[47] i.e., at the time when the cell-body changes its form both the cleavage-centrosomes and the supernumerary centrosomes simultaneously develop radiations

[47] Wilson describes this synchronism in the behavior of cytasters and division-asters in the egg of Toxopneustes (Archiv für Entwicklungsmechanik, 1901, vol. 12, p. 529): "It is a noteworthy fact that the whole cycle of changes involved in the division of the cytasters takes place nearly synchronously with those in the division-asters (p. 554)." Cf. also Kostanecki's observations on the parthenogenetic eggs of Mactra (Arch. f. mikr. Anat., 1908, vol. 72, p. 327). Also Conklin's studies on Crepidula eggs (Journal of the Academy of Natural Sciences of Philadelphia, 1912, vol. 15, section on Cyasters and Polyasters, p. 542). These structures appear to consist of the same material as cleavage-asters, and exhibit closely similar behavior; Conklin regards the cytasters as "isolated portions of archiplasm . . . . which take the aster form during mitosis and the vesicular form during resting periods;" in polyastral mitoses and similar conditions, "all asters within the same egg are in divisional activity at the same time."

about themselves, just as if each were an independent center of chemical activity influenced by some single change to which the entire cell responds. According to the present hypothesis, this inciting change is the surface-depolarization resulting from temporary increase of permeability. Many experimental facts are in harmony with this view. Wilson's observations on the abnormal eggs of Toxopneustes seem especially significant; he describes certain eggs which fail to divide, but in which nevertheless the nucleus and centrosomes pass through alternating periods of activity and rest similar to those of normal eggs;[48] the appearance of amœboid changes of form, indicating alterations of surface-tension, at the times when radiations develop, and their disappearance at the interkinetic periods when the nuclei reform and radiations disappear, indicate clearly an interdependence between surface-changes and the development of radiations. The influence of anæsthetics on these phenomena points in the same direction. Evidence from many sides indicates that the essential basis of anæsthesia in cells and irritable elements consists in an alteration of the plasma-membrane, rendering the latter resistant to changes of permeability and electrical polarization; hence stimulation and other effects dependent on changing polarization are prevented.[49] The fact that in both normal and abnormal eggs the development of radiations is prevented and existing radiations are suppressed by etherization,[50] is thus in harmony with the view that these phenomena—like the phenomena of stimulation—are a consequence of altered surface-polarization. We may infer that the plasma-membrane of the etherized egg is no longer capable of variations of permeability and polarization; and that for this reason the chemical changes in the centrosomes, normally initiated in this way, are no longer possible.

The facts of cytology must be considered in the light of results in other fields of cell-physiology—especially those relating to

[48] Archiv für Entwicklungsmechanik., 1901, vol. 12, p. 546.

[49] Cf. my review in Science, N.S., 1913, vol. 37, p. 959; also Amer. Journ. Physiol., 1913, vol. 31, p. 275, and Biological Bulletin, 1916, vol. 30, p. 311.

[50] Cf. Wilson, Phenomena of fertilization and cell-division in etherized eggs, Archiv f. Entwicklungsmechanik., 1901, vol. 13, p. 353.

the stimulation-process in its various modifications (conduction, inhibition, sensitization, anæsthesia, etc.). The essential conditions controlling intracellular processes are probably the same in all cells; of these conditions surface-processes are now recognized as all-essential; it is to be assumed therefore that they determine many phenomena of cell-division, as of other cell-activities. A somewhat more definite physico-chemical interpretation of the above phenomena seems desirable as a working hypothesis; and the following conception of the conditions within the dividing cell, partly a résumé and synthesis of the foregoing, seems consistent with the most general facts disclosed by cytological studies.

Apparently there exist in dividing cells certain localized reserves of oxidizable material; this material corresponds to the 'archiplasm' or 'kinetoplasm' of cytologists;[51] it appears to be present not only during division (when its quantity seems to increase), but in the interkinetic intervals also, although then usually difficult to detect; at the periods preparatory to division, when the assumed change of surface-polarization begins, it undergoes chemical change, e.g., oxidation—very much as do the carbohydrate reserves in muscle-cells—with the production of a diffusible electrolyte, e.g., acid; this, by its diffusion into the surrounding cytoplasm, creates about the active region (= centrosome) a local electrical field, corresponding to the diffusion-field; colloidal particles in this field become polarized, and frequently fuse to form radiating strands along the electrical lines of force. These local radiating areas, once formed, influence other cell-processes, e.g., the movements of formative or other materials; their influence on the distribution of chromosomes is evident, especially in polyas-

[51] Its chemical nature is uncertain, but there is evidence that it is derived from the nucleus; some of this material appears frequently if not usually to be introduced into the resting egg-cell by the spermatozoön in fertilization (as evidenced by the sperm-aster, hence Boveri's hypothesis); but apparently most is furnished by the egg, as indicated by the possibility of parthenogenetic development. Various facts indicate that the centriole consists of readily oxidizable (*i.e.*, strongly reducing) material; cf. the interesting paper of Mathews: Amer. Journ. Physiol., 1907, vol. 18, p. 102.

trial mitoses; they appear also to exert a direct influence on surface-tension, in the manner indicated below. Hence they undoubtedly form important accessory factors in cytoplasmic division, in addition to the primary factor of altered surface-polarization already considered.

This view regards asters (radiating areas in the cytoplasm), as temporary formations due to local chemical change, and hence accords with conceptions widely held among cytologists. Wilson describes "the rapid and complete disappearance of the rays upon cooling or etherization and their reappearance upon recovery;" this is intelligible, he says, "if the aster be only a radial configuration of the alveolar meshwork caused by an activity in the cytoplasm that is diminished or suspended by lowered temperature or etherization and renewed upon recovery."[52] Other cytologists express similar views. F. R. Lillie finds that when basophile particles, normally not present in the substance of the astral radiations, are artificially driven by centrifugalization into the spindle-area, they assume regular orientation along the strands and contribute to the formation of spindle and astral fibers.[53] "The center of force hypothesis offers the only consistent explanation of such behavior." These facts are plainly in harmony with the general conception that the centrosomes represent local accumulations of a special substance, 'archiplasm,' which influences the configuration of the cytoplasm only when it undergoes chemical change.[54] It seems also necessary to regard this substance as a product of cell-metabolism and colloidal in character; such a view explains the possibility of its continued production, and certain features of its behavior—e.g., the multiplication of centrosomes by division, the formation of supernumerary asters, the transport of kinetoplasm from cell to cell (as seen in the formation of sperm-asters in fertilization), and similar phenomena. It has been suggested above that the chemical change which it undergoes

[52] Loc. cit., pp. 384, 385.

[53] F. R. Lillie, Biological Bulletin, 1909, vol. 17, p. 101.

[54] This corresponds to Conklin's above-quoted conception of asters as representing isolated portions of archiplasm which assume the aster form during mitosis. Cytasters appear to be physiologically similar to cleavage asters, and to result from alteration of the same material.

at kinesis is an oxidation, resulting in the formation of acid products; this view agrees with the fact that cell-division is prevented by cyanide or lack of oxygen, and that polar and other radiations once formed are suppressed by cold, etherization, or exposure to boiled sea-water or a hydrogen atmosphere.[55] Mathews has put forward the hypothesis that the reason why aster-formation and cell-division are not possible in eggs until the germinal vesicle has broken down, is due to the necessity of some nuclear component, e.g., oxidase, for the oxidation-process in question. The cytological evidence that the centrosomal substance is of nuclear origin, or at least has a nuclear component, is very strong.[56]

The relation of astral formations to cytoplasmic division may now be briefly considered. These structures have long been regarded as especially concerned in the change of form of dividing cells; as "an expression of the forces operative in the division of the cell-body" (Wilson),[57] or as influencing the surface-tension of the cell (Conklin).[58] Enucleate cells containing asters may divide or undergo changes of form;[59] this happens especially when the asters approach the cell-surface; the region adjoining such an aster shows increased curvature, indicating increased surface-tension; cleavage furrows, and in some cases complete separation of portions of protoplasm, may result. The influence of astral areas in causing changes of form is most clearly seen in enucleate eggs, or in eggs containing supernumerary asters, or in which the conditions of the asters is artificially modified, e.g., by etherization; single asters may give rise to cleavage-furrows; their activity is greatest when they are near-

[55] Numerous instances of these effects are described in the already quoted papers of Wilson and Conklin. For the relation of oxygen to astral radiations cf. especially Mathews: loc. cit., p. 98.

[56] Cf. Mathews, Amer. Journ. Physiol., 1907, vol. 18, p. 94. The dissolution of the nuclear membrane is probable one of the changes resulting from the initial surface-depolarization of the cell, as suggested in my paper in the Journal of Morphology, loc. cit., p. 727; centriole-forming material would then be free to enter the cytoplasm.

[57] Loc. cit., p. 380.

[58] Loc. cit., p. 532.

[59] Cf. Wilson, Arch. f. Entwicklungsmech., 1901, vol. 12, p. 551; McClendon, ibid., 1908, vol. 26, p. 662.

est the surface; suppression of the radiations by etherization involves suppression of divisional activity; incomplete division is correlated with incomplete development of asters; in eggs containing several asters of varying sizes the depth of the furrow adjacent to each aster is proportional to the development of the latter; in eggs whose asters have been artificially suppressed by ether the rays may subsequently redevelop, and the effect on the form of the adjoining cell-surface is then in direct ratio to the development of the rays.[60] All of these facts indicate clearly the existence of an influence emanating from the aster, proportional to the development of the latter and its distance from the surface.

The hypothesis that substances diffusing from the astral centers reach the surface and there cause local lowering of surface-tension is naturally suggested by such facts as the foregoing. The evident correlation of this influence with the development of the radiations indicates, however, that the electrical factor, rather than the mere diffusion of surface-active substances, is the essential one. Moreover, the influence is apparently in the direction of increasing rather than decreasing surface-tension, since local increase of curvature in a fluid droplet implies increased tension,[61] i.e., traction toward that area. The hypothesis which seems to me most consistent with all of the facts considered is briefly as follows: the effect of the astral area in locally increasing surface-tension is due to the local electrical field, negative centrally, of which it is the expression; such a field, on approaching sufficiently near the cell-surface—of which the polarization is positive externally, negative internally—will compensate this polarization to a greater or less degree and hence increase the surface-tension. The periphery of the astral field is positive (due to the slight surplus of more rapid or more readily penetrating cations); we may therefore regard the astral areas as a source of cations (possibly hydrogen ions from CO_2, etc.), which increase the positivity of the protoplasmic layer immediately beneath the cell-surface, i.e.,

[60] Instances of all of these phenomena are described in Wilson's second paper, loc. cit.; cf. especially pages 367, 368, 372, 381.

[61] Cf. footnote 14; also Freundlich's Kapillarchemie, p. 212.

diminish the P.D. of the double layer at that region, to a degree which is greater, the nearer the astral area approaches the surface. The effect of the approaching astral field upon the polarized cell-surface is thus a local decrease of polarization, hence a local increase of surface-tension and an increased curvature of the surface.

Whether this effect is adequate to produce the results observed cannot be said definitely in the absence of quantitative data; there seems, however, to be no doubt that, granting the existence of an externally positive surface-polarization and a centrally negative astral diffusion-field, a qualitative effect of the above kind would be produced.

We may now briefly summarize the results of the foregoing discussion of the conditions of protoplasmic change of form. In normal mitosis cytoplasmic division is the consequence of a definitely localized surface-depolarization resulting in increased surface-tension. This effect is produced by the coöperation of two factors; one the general increase in the permeability of the cell-surface (assumed to be greater about the circumpolar or extra-equatorial areas), the other the depolarizing effect of an electrolyte diffusing from the chemically active astral centers. It is assumed that by the summation of these two effects the surface-tension is increased over the greater part of the area of each hemisphere sufficiently to account for the observed change of form. The relative importance of these two factors cannot be decided at present; it is indeed difficult to distinguish the parts played by each; probably some effect analogous to auto-stimulation also enters, the initial surface-change (due, e.g., to the spermatozoön) causing secondarily astral activity, and this again causing further surface-change—the two processes mutually influencing each other, like the local change of permeability and the bioelectric variation in the process of conduction of excitation.[62] Complete mitotic

[62] I.e., in the conduction of the excitation-state the original stimulus causes a certain local alteration in the surface-layer; apparently this originates a local bioelectric variation which acts as stimulus on adjacent regions, the original effect being thus extended and reinforced. For a fuller account of these conditions, cf., Amer. Journ. Physiol., 1914, vol. 34, p. 414, 1915, vol. 37, p. 348, and 1916, vol. 41, p. 126.

cell-division seems, however, to be unusual without the presence of definite centrosomes, so that the predominant rôle is probably to be attributed to the latter, the surface-change serving mainly to initiate their activity. Probably conditions vary considerably in different cells. It is interesting to note that conclusions in many respects similar to the foregoing were expressed by Wilson fifteen years ago; after pointing out that the influence of the aster on the cell-surface depends upon its distance from the latter, he continues: "it is altogether probable that a second factor lies in the physical changes taking place in the peripheral protoplasmic layer at the time of division, but this factor is itself probably dependent on the position of the aster, since we know that the direction of the cleavage-plane varies with that position."[63]

To a large degree any such hypothesis as the above is tentative, if not indeed chiefly valuable as a guide to research; it should, however, be noted that any theory of cell-division must of necessity involve the synthesis of a large number of data from different fields; further, that in endeavoring to form valid conceptions of vital processes the formation of such syntheses is indispensable. The dividing cell, like any other living system, has a unity which cannot be experimentally analyzed without the destruction of those vital properties whose elucidation is the very object of biological research; our only hope of understanding these properties lies in a reconstruction based on all known relevant facts and principles. Hence such reconstructions must be attempted, not only for guidance in research, but in the interest of complete knowledge.

SUMMARY

1. Sea-water of a dilution sufficient to cytolyze all unfertilized Arbacia eggs in half an hour or less, causes osmotic swelling but not cytolysis in uncleaved fertilized eggs (up to a few minutes before cleavage begins). At or about the time of formation of the cleavage-furrow a marked decline takes place in the resistance of the egg to hypotony, and cytolysis is then rapid and complete. When the cleavage-furrow is fully formed the origi-

[63] Loc. cit., p. 377.

nal resistance returns. A similar reversible decline of resistance takes place at the second and third cleavage, and is probably general for mitotic cell-division.

2. The minimum of resistance is found during the formation of the furrow. Both the decline and the return of resistance are rapid, the greater part of each phase occupying four to five minutes (at 22°). Some increase of susceptibility is apparent ten or twelve minutes before the first appearance of the furrow.

3. A decrease in the coherence or extensibility of the plasma-membrane at the time of cytoplasmic division is thus indicated. The earlier observations of Lyon and other investigators have shown that an increase of susceptibility to poisons, heat, and other injurious conditions, together with an increased output of CO_2, takes place at this time, i.e., simultaneously with this change in the membrane. The above facts constitute additional evidence that an intimate connection exists between the general physiological condition of the egg and the physical state of the plasma-membrane.

4. The above change in the membrane is probably associated with an increased permeability to water-soluble substances and a decreased electrical polarization; this latter change, according to the law of electrocapillarity, involves increased surface-tension. From the analogy with the general stimulation-process, it seems also probable that the change of polarization acts upon the conditions within the dividing cell (oxidations, etc.) in a manner analogous to the similar change in electrical stimulation in general.

5. The following hypothesis of cytoplasmic division is put forward. The change of form is the result of two chief factors: (1) a definitely localized increase of surface-tension, resulting directly from increased permeability and decreased electrical polarization of the cell-surface, over two symmetrical areas centering at the poles and extending to near the equator; and (2) a secondary or adjuvant effect of the same kind due to the diffusion of electrolytes (e.g., acid derived from oxidations) from the astral centers or centrioles, which become chemically active at this time. These centers appear to represent aggregations of a special colloidal material which undergoes oxidation when the cell-surface undergoes depolarization.

THE ABSORPTION OF NUTRIMENT FROM SOLUTION BY FRESHWATER MUSSELS[1]

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THIRTY FIGURES (TWO PLATES)

CONTENTS

INTRODUCTION

In 1914 the author began a series of investigations upon fresh-water mussels designed to ascertain whether or not there could be found any direct proof, especially of a histological nature, of the correctness of Pütter's theory that animals living

[1] Published by permission of the Commissioner of Fisheries.

in the water use, in addition to 'formed' food, nutriment which is in solution in the surrounding medium. He wished also to put to the test Pütter's assumption that some of such nutriment is absorbed directly by the cells of the outer body walls, especially by those of the gills.

Pütter ('06) was the first seriously to advance the theory that food could be so taken. He based his conclusions in part on a comparison of the amount of carbon necessary for the maintenance of the organism with the amount furnished by the plankton. Pütter considered the latter too small for the needs of the animal and urged that it must use some carbon which is in solution in the water, resulting from the decay and disintegration of organic life. He further stated that the amount of material found in the alimentary canal is never large enough to supply the requisite quantity of carbon. Besides the alimentary canal, the uncutinized epithelium of the outer surface of the body, especially that of the gills, was thought to function in absorbing dissolved food. This process was considered to go on in addition of course to the digestion of 'formed' food by the alimentary canal. Pütter concluded that the above conceptions applied to Protozoa, Porifera, Echinoderms, Crustaceans, Mollusks and Fishes. He tested the matter experimentally in two ways: first by noting that goldfish and perch lived longer in solutions of asparagin, somatose and glycerin than in tap water: secondly by comparing the amount of oxygen needed to oxidize the lost weight of tissues of actinians, tunicates and fish while kept in their natural medium, with the amount of oxygen actually used. As the latter was found to be greater than the estimated quantity needed he concluded that the extra oxygen was used in oxidizing some food that had been taken from the water where it had been present in the form of a solute.

Lohman ('09) estimated the amount of plankton found in sea-water from various parts of the ocean and concluded that plankton comprised the main source of the food of aquatic forms. He quoted Henze's researches as showing that there is present in sea-water no appreciable amount of carbon compounds in solution.

Knorrich ('10), working with Daphnia, which lived 14 days in sterilized hay solution, concluded that nutriment was absorbed from the solution.

Kerb ('10), kept eels in sugar solutions and noted no diminution in the amount of sugar from day to day. He obtained similar results while working with Corethra larvae in sugar solutions. Also he found that Daphnia lost in dry body weight as rapidly in solutions of peptones as in tap water.

Wolff ('10), working with Simocephalus, found that it lived twice as long in bacteria-free water, which contained some dissolved carbon compounds, as it did in tap water. He made no observations as to body weight lost or gained.

Several American investigators of the question of the nutrition of the oyster have felt that the amount of material found in the alimentary canal was not large enough to account for the growth of the oyster to the relatively large size which it attains in the first two years of its existence.

Grave ('12) in particular suggested that in the oyster food may be taken in by means other than that of the alimentary canal.

Moore, Whiteley, Edie and Dakin ('12) investigated the rate of oxidation and the output of carbon dioxide by aquatic invertebrates in relation to the available food supply in sea-water. They also made chemical analyses of samples of sea-water from various regions. Their general conclusion was that sea-water does not contain any appreciable amount of dissolved organic matter capable of acting as a nutrient medium for animals living in it.

Lipschütz ('13) reviewed almost the entire subject including his own previously published experiments along that line and offered criticism of Pütter's work. Lipschütz noted that fish and eels when kept in nutrient solutions lost as much body weight as in tap water. He also thought Pütter had overestimated the amount of material in solution in the water and underestimated the carbon content of the plankton. His general conclusions are the opposite of those of Pütter.

Blegvad ('15) found that the invertebrates on the sea-bottom in Danish waters live on detritus and to a lesser extent on plankton. He does not corroborate Pütter's theory but on the contrary thinks that the latter did not allow enough for the quantity of detritus.

Esterly ('16) found that in pelagic copepods the amount of food that is indicated by the intestinal contents is 'surprisingly small.' He suggests however that it is probable that much of the food of these forms consists of organisms without shells and which do not leave recognizable remains in the intestine. Further careful investigation he feels is necessary before Pütter's theory is accepted.

Lund[2] found that if Bursaria are kept in a weak soap solution they will absorb fat from such solution through their body walls.

The present author, in 1915, published a paper in which were set forth the results of the earlier steps of his investigations. Fat was selected for the initial experiments as it is most easily traced histologically. Following the method of Lund, olive oil was saponified and the mussels kept in weak solutions of the resulting soap. In some cases the solutions were stained with Sudan III. Briefly stated, it was found that the mussels absorbed fat from the solutions to a marked extent. This was transported over the body by the plasma of the blood and by the corpuscles. Several of the experiments went far toward proving that some of the fat was absorbed directly by the cells of the gills, mantles and foot.

The present paper discusses the results of the continuation of the series of investigations. Further work was done on the absorption of fat using a commercially prepared soap instead of the saponified oil. The absorption of protein was studied somewhat extensively and some efforts were made to ascertain the facts in regard to the absorption of starch.

The author wishes to express his obligations to Dr. Caswell Grave, at whose suggestion the work was undertaken, for advice and aid given throughout the course of the investigations:

[2] Dr. Lund's results are not yet published

to Dr. H. S. Jennings and Dr. R. E. Coker for their suggestions and interest in the work: to Dr. G. L. Houser for library facilities at the University of Iowa; and to Mr. A. F. Shira, Director of the Biological Station at Fairport, Iowa.

MATERIALS AND METHODS

The mussels upon which the investigations were carried out were individuals selected from the more common species found in the Mississippi River near Fairport, Iowa. Adult specimens were employed in the following experiments. Care was exercised to choose non-gravid mussels which were in a seemingly healthy condition, 'shoulder-raked' or hand collected individuals being used in preference to those dragged out of the water by 'crow-foot' hooks.

At Fairport soft water was used for the work with fat, for the reason that the soap was more soluble in it than in river water. For the experiments with protein and starch filtered river water was used. At the Johns Hopkins Laboratory, where the work concerned only protein and starch, either city tap water or Chattolanee spring water was used.

The mussels experimented with were kept in glass aquaria of about 5000 cc. capacity. Control individuals in filtered water were kept side by side with those in the solutions. The solutions were changed daily in most cases. In some of the experiments with protein the changes were made twice daily. By this procedure the protein was prevented from undergoing marked decomposition. As the mussels are accustomed to a current of water this method of manipulating the aquaria seemed to be the closest approximation to natural conditions which it was possible to obtain in the laboratory.

For the experiments with fat a 'non-alkaline' soap commercially prepared from olive oil was used. This was done to avoid the free alkali and oil present in the saponified olive oil employed in the earlier investigations. The strength of the solution used was 0.002 of one per cent. The tissues of various portions of the mussels experimented with were sectioned by the freezing

method, stained with Sudan III and mounted in glycerin. In some cases the soap solution was stained by dissolving Sudan III in it to the point of saturation. Sections of mussels which had been kept in such solutions were mounted directly after cutting.

A method of attacking the question of the absorption of protein was much more difficult to devise. Egg albumin was chosen as the most convenient protein with which to work. The white of one egg was stirred into 300 cc. of water and the mixture filtered. The soluble albumin passed through the filter leaving the insoluble globulin behind. (The presence of albumin in the filtrate could be demonstrated by the formation of a white precipitate on the addition of alcohol.) 50 to 100 cc. of the filtrate were used in 5000 cc. of water to make the solutions in which the mussels were kept. Some experiments were continued for considerable periods of time, thirty to sixty days, and histological evidence sought of differences in the condition of the cells of those individuals which had been in the solutions and of the control mussels. As the latter, being in filtered water, received little food it was thought that evidence of starvation might be detected by the appearance of the cells. In other cases the experiments were continued only a few days, the tissues of the mussels were fixed in absolute alcohol or other fixing fluids and sectioned in paraffin. These were studied both stained and unstained. Efforts were made to find a specific stain for the albumin. Cowdry ('14) has found that Janus green, besides staining mitochondria, stains albumin in the living cell. This fact was made use of and mussels were kept in solutions of albumin stained with Janus green. Sections of such individuals were made by the freezing method and mounted in glycerin. The paraffin method failed with such sections as the stain is washed out very readily by the alcohols. It was necessary to study the sections mounted in glycerin within the course of a day or two as the stain is also removed by the glycerin in a short time.

Soluble starch was made by boiling and weak solutions were used in which to keep the mussels. Sections of such mussels

were prepared both by the paraffin and by the freezing methods and efforts were made to detect starch in the cells by staining with iodine. Mussels were also kept in solutions of starch which had been colored blue with iodine. Spring water was used in this case as the tap water contained so much free chlorin that the blue color was removed from the solution.

Further details of the methods employed will be stated in connection with the observations recorded below.

OBSERVATIONS

Fat

The solutions made from the commercial soap were found to be more satisfactory than those used in the previous work with fat where olive oil was saponified with sodium hydroxide. In the solutions from the latter the mussels were found to throw off much mucus, no doubt owing to the free alkali present due to a slight excess of sodium hydroxide employed in the process of saponification..

The mussels threw off no appreciable amount of mucus while in the solutions prepared from the non-alkaline commercial soap. None died while in the solutions. There was no evidence of any toxic effect due to the soap solution.

The results were the same as those obtained in the previous work with fat. The mussels which had been kept in the solutions were found to contain much more fat than those not so kept. This fat was distributed in the form of droplets[3] in greater or less abundance in nearly all parts of the body. Figures 1, 2 and 3 represent portions of a specimen of an adult Quadrula ebenus which had been allowed to remain in a 0.002 of one per cent soap solution for five days. The fat, represented in the drawing by the heavy black dots, may be seen in the epithelial cells of the gill filaments, the ostia, the water tubes and in the corpuscles.

[3] The term 'droplets' will in this paper be applied to the spherules of absorbed fat found in the tissues of the mussels experimented upon. These droplets usually were of diameters varying from less than one micron to 3 or 4 microns, though instances were found in which the diameter was as great as 7 or 8 microns.

Mussels were kept in soap solutions stained with Sudan III. A gill filament of such a mussel after it had remained in the solution for five days is shown in figure 4. Pink or orange colored fat droplets were found in great abundance in the epithelial cells of the filaments, ostia, water tubes and in the corpuscles. This fact furnishes direct proof that the heavy loading of fat found in the mussels which had been in the soap solutions was not due to the chance use of an extraordinarily fat individual. The colored fat could have come only from the stained soap solution.

Mussels with the valves wedged open were suspended over the solutions in such a manner that only the lower edges of the mantles were immersed in the solutions. The animal soon relaxed the foot so that the end of that organ also was covered. Control individuals were suspended in like manner over filtered water. The experiment was continued for forty-eight hours. Numerous fat droplets were found in the cells of the parts of the mantles and foot which had been immersed in the solution and none were discovered in similar tissues of the controls. Epithelial cells of the mantles and foot that had been covered by the solution are represented by figures 5 and 7, while figures 6 and 8 show cells of corresponding parts of the control muscles.

It was thought possible that in this case the presence of fat was due to fatty degeneration incident to the weakened or moribund condition in which the mussel might be supposed to be after having been suspended thus with the larger portion of its body in the air for forty-eight hours. Such a supposition was rendered negligible by the facts that the mussels in which the fat was found were apparently in a healthy condition when killed; that no fat was found in the controls and none in mussels which had been allowed to die while so suspended over filtered water.

Finally, a large mussel with the valves wedged open was placed over the edge of the aquarium in such a manner that the lower edge of one mantle only was immersed in the soap solution, the entire remainder of the body being in the air. After a period of forty-eight hours sections of the part of the mantle

which had been in the solution revealed many fat droplets in the cells on the side next to the body. No fat droplets were found in the cells of the corresponding part of the other mantle which had not been in the solution. Some of the cells of the mantle which had been in the solution are represented in figure 9, while figure 10 shows cells of the other mantle which had remained in the air. In all these cases in which the mussels were suspended in such a manner as to allow only a portion of their body to come into contact with the solution, the oral and anal openings were above the solution so that none of the solution could have entered the alimentary canal directly. In the case last mentioned, in which one mantle of the mussel was immersed in the solution and compared with the other mantle of the same mussel, this mantle not having been immersed, direct proof of the absorption of fat from the solution by the epithelial cells of the mantle is given. In all cases in which one mussel is used as a control for another the objection can be raised that by chance a naturally fatter individual was chosen to be kept in the solution. But that objection can not hold in this case as it is hardly supposable that corresponding parts of the two mantles of the same mussel would differ in fat content as markedly as indicated in figures 9 and 10.

Protein

The problem of the absorption of protein was first attacked by means of the 'starvation' method. Five mussels were kept in solutions of egg albumin and five in filtered water. Both experiments were started on the same day and at intervals one individual was removed from the solution and one from the water at the same time and their tissues fixed and sectioned. Bouin's fixing fluid was used for this work and the sections were lightly stained with haematoxylin and eosin. One pair of mussels was killed at the expiration of ten days, other pairs at the end of thirty, forty-five and fifty-eight days. One of the individuals in filtered water died on the twentieth day. The tissues of the mussels were compared by the study of the sections for the purpose of discovering any possible difference in the degree

of emaciation between those that had been in the solution and those that had not. The criteria taken into account in judging the degree of emaciation were shrinkage of the cells and loss of capacity of the cytoplasm of taking up the eosin stain. Many workers on starved tissues have described great shrinkage and loss of staining capacity on the part of fasting cells. Morgulis, Howe and Hawk ('15) found that the cytoplasm of the cells of a dog which had suffered extreme starvation took a much less pronounced eosin stain than the cells of normal tissues.

The cells of the mussel which had been in filtered water for ten days showed no appreciable signs of emaciation. Various portions of its tissues were compared with corresponding parts of those of the individual from the solution, special attention being given to the epithelial cells of the gills in all the starvation experiments. In making the tests with the eosin stain a slide holding the sections from the mussel which had been in the solution was placed back to back with a slide of the sections of the control and both were passed through the alcohols and eosin together. In this way it was certain that both were exposed to the action of the reagents for exactly the same length of time and that any difference found in depth of stain was due to a difference in the cells and not to a variation in the time the tissues had remained in the stain. In some cases sections from mussels to be compared were mounted on the same slide.

In the comparison of the mussels killed at the expiration of thirty days, marked differences were found between the cells of those that had been in the solution and of those which had been in filtered water only. These differences were especially noticeable in the cells of the gills. The cells of the mussels which had been in the solution were plump, unshrunken and took the eosin stain in a normal manner. The gill filaments of those from the filtered water were small and wrinkled. The cells were shrunken and took a much lighter eosin stain than did those of the mussels from the solutions. Figures 11 and 12 represent respectively filaments of the mussel which had been in filtered water and of the one which had remained in the solution of albumin. These figures show most clearly the shrinkage

and distortion of the cells, the differences in the depth of stain taken being less exactly represented by the stippling. In figures 13 and 14 are shown respectively filaments of a mussel which had been subjected to filtered water and of one which had been in the solution for fifty-eight days. The same differences were found in this case as in the former. The pair killed at the end of forty-five days also manifested these differences.

The above facts leave little doubt that the mussels in the solutions make use of the albumin as food. The mussel that died during the course of the experiment was one of the lot in filtered water and its death was probably due to starvation.

Efforts were made, however, to devise methods whereby the albumin might actually be seen in the cells. Experiments were carried out in which some mussels were kept in solutions of albumin and others in tap or spring water. Sections of those in the albumin solutions were mounted on slides with corresponding sections of individuals which had been in water only. These were stained with eosin or Bordeaux red and a study of the minute structure of the cytoplasm was made. In general the cytoplasm of the mussels which had been in the solutions was of a more granular nature than that of the other mussels. The granules were not only more numerous but many of them were larger. For example, in one case where 100 cc. of albumin in 5000 cc. of water had been used in an experiment of twenty-one days' duration the difference was quite marked. The effect of the fixing agents is to precipitate the proteins of the cell. Apparently there was a greater quantity of protein present in the case of the mussels which had been in the solutions of ablumin.

In an effort to discover whether or not the same result could be obtained if the alimentary canal were eliminated, the mouths of some of the mussels were plugged with dumbbell-shaped pieces of paraffin. In many cases the mussels died due to injury incident to the operation. If no injury was inflicted the mussels often lived for a week or two. If the mussel did not die in the course of one or two days it might be assumed that no injury had been inflicted and that the mussel would live for at least eight days, which was the length of time it was desired

that the experiments should continue. The cytoplasm was studied after staining with eosin or Bordeaux red with the same results as in the previous experiments. The cytoplasm of those mussels which had been in the albumin solutions was more granular, the granules being more numerous and larger, than in the mussels which had remained in water only. This was especially apparent in the cases of two pairs of mussels from an experiment extending over six days. The solution in which the mussels had been kept contained 100 cc. of albumin in 5000 cc. of water. The cytoplasm was stained, in the case of one pair of mussels, with eosin and in the other with Bordeaux red. In both cases there were more granules in the cytoplasm of the mussels which had remained in the solutions than in that of the others. If the presence of a greater quantity of granules in the cells of the mussels which had been in the albumin solutions may be taken as evidence of the presence of albumin, in these cases in which the mouths of the mussels were plugged the albumin could have been taken up only by direct absorption from the solution.

Considerable effort was expended in attempting to discover a specific stain for the albumin, so that its presence might be detected in a manner similar to that by which fat is revealed by means of Sudan III. Albumin was precipitated with absolute alcohol, caught on filter paper and mounts made of it, both while wet from the alcohol and also after dehydrating, clearing and embedding in paraffin. While unstained, the appearance of the albumin under the microscope was that of a quantity of granules of various sizes. Most of them were irregular in shape but now and then one of a spherical form was discernible. Some of the mounts were carried through one stain, some through another, the object being to find a dye that would stain albumin and yet not stain the general cytoplasm of a cell. No great degree of success was attained. The albumin took the various stains tried, including eosin, Bordeaux red, haematoxylin, Janus green and others. All of these dyes stain the cytoplasm of fixed cells except haematoxylin, which is not considered a cytoplasmic stain. Since, however, the only color this stain im-

parted to the albumin was that of a weak bluish-brown it proved to be of no great value. When fixed tissues of the mussels which had been in the albumin solutions were treated with haematoxylin, it was found impossible to distinguish any granules in the general mass of the cytoplasm that might have been stained with the haematoxylin. The cytoplasm in unstained fixed tissues is of a brownish color and it was difficult accurately to determine the color of the small opaque granules found in these sections.

Albumin solutions were stained by dissolving haematoxylin in them. Mussels were kept in such solutions and control individuals were allowed to remain in tap water in which haematoxylin had been dissolved. The mouths of the mussels were plugged as described above. The experiment was continued for five days. At the close of that time portions of the gills were fixed in absolute alcohol and embedded in paraffin. As absolute alcohol precipitates albumin and does not dissolve haematoxylin from tissues, it was considered a favorable fixative for this purpose. The sections were mounted unstained and search was made for blue granules which might have been taken up from the solution by the cells. The results of such a study were of a negative nature. The same difficulty was experienced as described above in regard to the fixed tissues. While some of the granules in the cells which had been in the solutions seemed to have a bluish tint, in general it was impossible to be certain of the matter. In other words, haematoxylin does not impart to albumin a stain sufficiently characteristic to permit of its being distinguished from the unstained granules about it.

However, another result was obtained which confirmed the results of an earlier experiment. The cytoplasm of the cells of mussels which had been in the solutions was of a much more granular nature than that of the cells of the individuals which had not. The granules were both more numerous and larger. Also, many could be seen adhering to the outer ends of the cells or entangled in the cilia. Appearances indicated that these granules were particles of albumin and that some had been taken into the cells. The gill filaments of mussels which had

been kept in albumin solutions thus stained are represented in figures 17 and 18. Figure 19 is a representation of the gill filaments of a mussel which had remained in water in which haematoxylin had been dissolved. The granules in the latter will be seen to be much less numerous than in the former and none can be observed clinging to the outer ends of the cells or to the cilia.

Finally a fact was discovered that led to more definite results. Cowdry, in a paper on mitochondria, makes mention of the fact that he has found that Janus green will, in the living cell, stain lecithin and albumin, the latter more heavily. As mentioned above the author had found that in fixed preparations Janus green stained the entire cytoplasm. An attempt was now made to determine whether or not Janus green might be employed as a means of detecting albumin that might have been absorbed by the cells of the gills or other parts of the outer body walls of the mussels.

Mussels were put into solutions made up 5000 cc. of water and 100 cc. of albumin stained with Janus green. Control individuals were placed in water in which Janus green had been dissolved. The mouths of these mussels were left open. The experiments were continued for eight days. At the expiration of that period the mussels were killed and their tissues were hardened in 10 per cent formalin for a few hours. As albumin is precipitated by formalin, this procedure would cause the precipitation of the albumin in any of the solution which had adhered to the outer surfaces of the cells in addition to the coagulation of what albumin might have been absorbed. In this way aggregates of albumin sufficiently large to be visible under the microscope would be formed and thus render it possible to trace this protein histologically. Sections were then cut by the freezing method and mounted in glycerin. The gill filaments of a mussel that had been in the stained solution are represented in figure 20. Numerous green granules of various sizes were found within the cells and within corpuscles in the blood vessels in the center of the filaments. The granules which had taken the stain are represented in the drawing by heavy black dots. In

some cases these green bodies were almost spherical. This was especially true of the larger granules. In general the diameter of the granules varied from less than one micron to that of three or four microns. Green colored granules were also found clinging to the outer ends of the cells or adhering to the cilia. Figure 21 shows the filaments of a mussel kept in the water and Janus green. In this case no green granules were found either within the cells or on their outer surfaces. In some cases, both in the mussels that had been in the solutions and in the controls, the entire cytoplasm of the cells of the outer ends of the filaments showed a pale green tint. The granules in question, however, took a much deeper stain and were readily distinguishable from the surrounding cytoplasm.

Similar experiments were carried out in which the mouths of the mussels were closed with paraffin plugs. Exactly the same results were obtained. In figures 22, 23 and 24 are shown gill filaments of a mussel which had been in the stained solution. The black dots represent green granules and spherules which were found within the cells and corpuscles and clinging to the outer ends of the cells. The mussels used as controls in these experiments had no definite green granules within the cells or on the outer ends, though the cytoplasm often exhibited a faint green tint, as did that of those which had been in the stained solutions. A drawing of the gill filaments of one of the control mussels would present the appearance of the filament shown in figure 21. The presence of the green tint found in some cases over the entire cytoplasm does not militate against the efficacy of the Janus green as a specific stain for the albumin. Sudan III, while it is a specific stain for fat, often imparts a reddish tint to the entire cytoplasm, but stains the fat droplets a much deeper hue.

Figures 25, 26 and 27 represent respectively portions of the palp, foot and mantle of a mussel which had been in the stained solution. The mouth of the mussel had been closed with a paraffin plug. Green granules were found in the epithelial cells of all three tissues.

The above results demonstrate that the cells of the outer body walls of the mussels have the power to absorb at least one protein, albumin, from the surrounding solution.

Starch

Soluble starch was made by boiling commercially prepared cornstarch in water. One gram of starch was used in about 400 cc. of water. The solution, after boiling, was allowed to settle and 50 or 100 cc. of the supernatant liquid used in making the solutions in which the mussels were to be kept. This method affords only a rough estimate of the amount of starch present, as the amount of starch that went into solution was not determined. However, qualitative results only were sought in these initial experiments. The supernatant liquid was proved to contain starch by testing with iodine. After the mussels had remained in the solutions for various lengths of time they were killed and sections of their tissues were made both by the freezing and by the paraffin methods. The sections were then placed in alcoholic solutions of iodine for different periods of time and an effort was made to find blue granules within the cells. The results were negative. The tissues as a whole often assumed a slight yellow tint from the influence of the iodine, but no granules were found that could be considered to have a blue color. As iodine stains dextrin or causes it to assume a red color, search was made for red particles in the cells. It was thought possible that the starch might have been more or less completely converted to sugar by the cells and that evidence of some of the incidental steps could be found. Very small red granules were found both in the tissues of the mussels which had been in the solutions and in those of the controls. These red particles were no doubt the pigment granules which impart to the tissues the pinkish hue observable in many specimens of Quadrula ebenus. They presented no difficulties in the work with fat which had been stained red with Sudan III, as the pigment was in the form of small, irregular, highly refractive granules readily distinguishable from fat droplets. Their presence offered some

complications, however, in the search for any red granules that may have been present owing to the conversion of starch to dextrin and to its subsequent staining with iodine. No satisfactory evidence of the absorption of starch was disclosed by the above methods.

The experiment of keeping the mussels in a starch solution which had been stained blue with iodine was then tried. The mouths of the mussels were plugged with pieces of paraffin as described above. After the expiration of four days the mussels were killed and sections were made of their tissues by the freezing method. The sections were mounted at once in glycerin without staining. In the cells of the gill filaments some granules were found that were blue in color, though the observation was made with difficulty owing to the opacity of the granules. Similar granules were found within the corpuscles and also clinging to the outer ends of the cells and to the cilia. They were quite irregular in shape and varied from one to three microns in diameter. The gill filaments of a mussel which had remained in the stained starch solution for four days are represented in figures 28 and 29. Figure 30 is a representation of the gill filament of a mussel kept in tap water.

These results present some evidence that starch may be taken up directly from a starch solution by the epithelial cells of the gills of the mussel. Since fewer experiments were carried out with starch than with fat and protein, the results can not be considered to be as conclusive as were those attained in the work with the latter.

Behavior of the corpuscles

An interesting point in connection with the behavior of the corpuscles of the blood was noted. In the author's previous paper dealing with the absorption of fat, mention was made of the fact that the corpuscles were often found pressed closely against the bases of the epithelial cells of the gills and that the corpuscles carried some of the fat droplets from the cells to the other parts of the body. Some of the fat droplets were appar-

ently transported while floating in the plasma of the blood. A description has been given above of the discovery of fat droplets, granules of albumin or of starch, as the case might be, in the corpuscles of the mussels which had been in the solutions. It was also found that the corpuscles, in addition to pressing closely against the bases of the cells of the gills, actually wandered out between the cells in such a manner that their entire surfaces were in touch with the cells, the corpuscle lying in a sort of cup between the contiguous cells. This phenomenon is illustrated in figures 15, 16 and 17. In great numbers of cases the corpuscles were found pushed out between the cells in such a way as to be entirely surrounded. In other instances a corpuscle might be observed partially surrounded as in figure 15, the process of fixation having killed it at the moment of entrance to or exit from between the two cells. Undoubtedly a position in which the corpuscle is entirely surrounded by the epithelial cells affords more surface for the exchange between the cells and the corpuscle not only of materials concerned in respiration, but also for the reception by the corpuscle of food material absorbed by the cells of the gill. Similar behavior of the corpuscles was not noted in parts of the body other than the gills.

SUMMARY AND DISCUSSION

The above results leave no doubt of the fact that mussels may make use of some kinds of food which are in solution in the water. A part, probably a small one, of such nutriment can be taken up directly by the outer epithelial cells of the body. As these animals are provided with a well developed digestive apparatus we may suppose that the absorbing power of these outer epithelial cells is a property that has been retained from a more primitive state in which the cell was less highly specialized and that this property is not a special adaptation correlated with the lack of a functional digestive system.

The nutriment taken up from the solutions by the mussels by means of the alimentary canal was no doubt absorbed by the cells lining the intestine in the usual manner in which 'formed'

food is absorbed after being rendered soluble by the digestive juices. In regard to the mechanism of the absorption of the foods by the cells of the outer body walls little can be offered.

In the case of the fats, numerous droplets which took the Sudan III stain were found closely attached to the outer ends of the epithelial cells of the gills or mantle. These droplets were probably the fatty acids, a small part of which were present in the soap solution owing to hydrolysis, by which process sodium hydroxide and the fatty acids would be formed; the remaining droplets were no doubt due to a slight acidity of the surface of the living cells resulting from the union of carbon dioxide from the cells with the water, forming carbonic acid. In the experiments in which olive oil was saponified no attempt was made to remove the glycerin from the mixture. Therefore free fatty acids, from the droplets just mentioned, and glycerin may have been absorbed separately and resynthesized to fat within the cells. This absorption may have been effected by phagocytic or amoeboid action of the cells or by solution in the plasma membrane and reprecipitation within the cell. In the case of the commercially prepared soap the objection might be raised that there was no glycerin present to be absorbed and reunite with the fatty acids after their entrance into the cells. However it is safe to assume that a moderate amount of glycerin was present in the soap from the fact that in the process of its manufacture the soap is 'salted out' of the glycerin, allowed to rise to the surface and removed while wet with the glycerin. Not all the glycerin is removed by the subsequent drying.

Again it is not certain that the drops taking the osmic acid stain as described in the author's previous paper or the Sudan III stain in the sections prepared by the freezing method were fats or only the fatty acids. It is known that osmic acid blackens or browns free fatty acids. It is possible that Sudan III also stains them. At any rate solutions made from the commercially prepared soap dissolve Sudan III readily and assume the characteristic red color which that stain imparts to fat. It is probable that both these stains for fat really affect the fatty acid radical only and that it is that radical which carries the Sudan

III into the cell. Therefore there may exist the possibility that the sodium oleate, stearate and palmitate from the soap may have entered the cell as such, the radical carrying the stain. The sodium may then have been separated leaving the radical free to unite with any glycerin which may have been absorbed.

In regard to the absorption of albumin it is necessary to assume either a power on the part of the cell to split the protein into its amino acids and the absorption of these as in the alimentary canal, or the direct taking in by the cells of the colloidal particles of albumin by means of something analogous to phagocytic action. The fact that the Janus green stain was carried into the cell offers some evidence that the albumin entered the cell as such without being previously split into the amino acids.

In the case of starch it seems probable also that the granules entered the cell by amoeboid or phagocytic action. The presence of the definite blue granules within the cells would somewhat oppose the theory of any conversion of the starch previous to absorption.

The present investigations demonstrate only the ability of the mussels to make use of nutriment which is in solution in the water by the twofold means cited above. They do not deal with the possible amount of nutriment present in various bodies of water in which aquatic animals are found. The investigations show that if dissolved material is present the mussels can make use of it. After the ability of the animal to make use of food in such form is proved, the question of whether or not, in any particular case, it does do so, depends upon the presence or absence of such food in the water. It is too sweeping a statement to assert that aquatic animals in general do not or can not make use of nutriment which is in solution in the water merely because no dissolved compounds are found in certain analyses of water taken from a more or less limited region. Nor is the fact of the presence of an amount of detritus, apparently adequate for the nourishment of a bottom-living animal, sufficient proof that all its nutriment is made up of such detritus.

In the case of mussels in particular it is very probable that considerable nutriment is in solution in the water in which they live. While no analysis of the water has been made thus far in the investigations, the Mississippi River, as everyone knows, drains a vast area of land from which refuse from decaying animal and vegetable material is collected in very great abundance. The dead bodies of aquatic forms of many sorts add to this supply. The mussels are bottom-living organisms and come into contact with decaying and dissolving organic matter which is lying on or being slowly moved along the substratum. Solutions or colloidal suspensions of the proteins must certainly be present in some abundance. As the water is slightly alkaline some of the fat from decaying organisms is probably saponified and thus distributed throughout the water instead of rising to the surface where it would be inaccessible to many forms.

In general the question of whether or not a particular aquatic animal absorbs nutriment from solution by means of the alimentary canal and the outer body walls probably depends on the presence or absence of dissolved substances in the water in which the animal in question lives. Fresh-water mussels can absorb nutriment which is in solution in the water and it seems very probable that other forms likewise possess this ability.

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PLATES

EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida; Leitz Oc. No. 4, Obj. No. 6 unless otherwise indicated.

PLATE 1

EXPLANATION OF FIGURES

1 Gill filament of Quadrula ebenus which had been in soap solution for five days. *A*, fat droplets; *B*, blood corpuscles containing fat droplets; *C*, chitinous rod.

2 Section from the same mussel as in figure 1, showing fat droplets in epithelial cells of *A*, ostium.

3 Epithelial cells of water tube of gill of same mussel as in figure 1.

4 Gill filament of Anodonta imbecillis which had been for five days in soap solution which had been stained with Sudan III. *A*, pink colored fat droplets; *B*, blood corpuscles containing pink fat droplets; *C*, chitinous rod.

5 Epithelial cells of mantle of Lampsilis ventricosa which had been suspended for forty-eight hours in such a manner that only the extremity of the foot and mantles were immersed in soap solution.

6 Epithelial cells of mantle of the control for figure 5, the mussel being suspended so that the foot and mantles were in water.

7 Epithelial cells of foot of the same mussel as figure 5.

8 Epithelial cells of foot of same mussel as in figure 6, control for figure 7.

9 Epithelial cells of left mantle of Lampsilis ventricosa. These cells are from the edge of the mantle which was immersed in soap solution, the remainder of the mussel being in the air.

10 Epithelial cells of right mantle of the mussel shown in figure 9. These cells were not exposed to the solution.

11 *A*, *B*, and *C*, gill filaments of Quadrula ebenus which had been in filtered water for thirty days.

12 Gill filaments of Q. ebenus which had been in albumin solution for thirty days. The relatively heavier stippling in this drawing in comparison with that in figure 11 indicates the difference in depth of eosin stain taken by the cells of the two mussels.

28 and 29 Gill filaments of Q. ebenus which had been for 4 days in starch solution stained blue with iodine. Mouth plugged. *G*, blue granules of starch.

30 Gill filament of Q. ebenus which had been in water only. Control for figures 28 and 29.

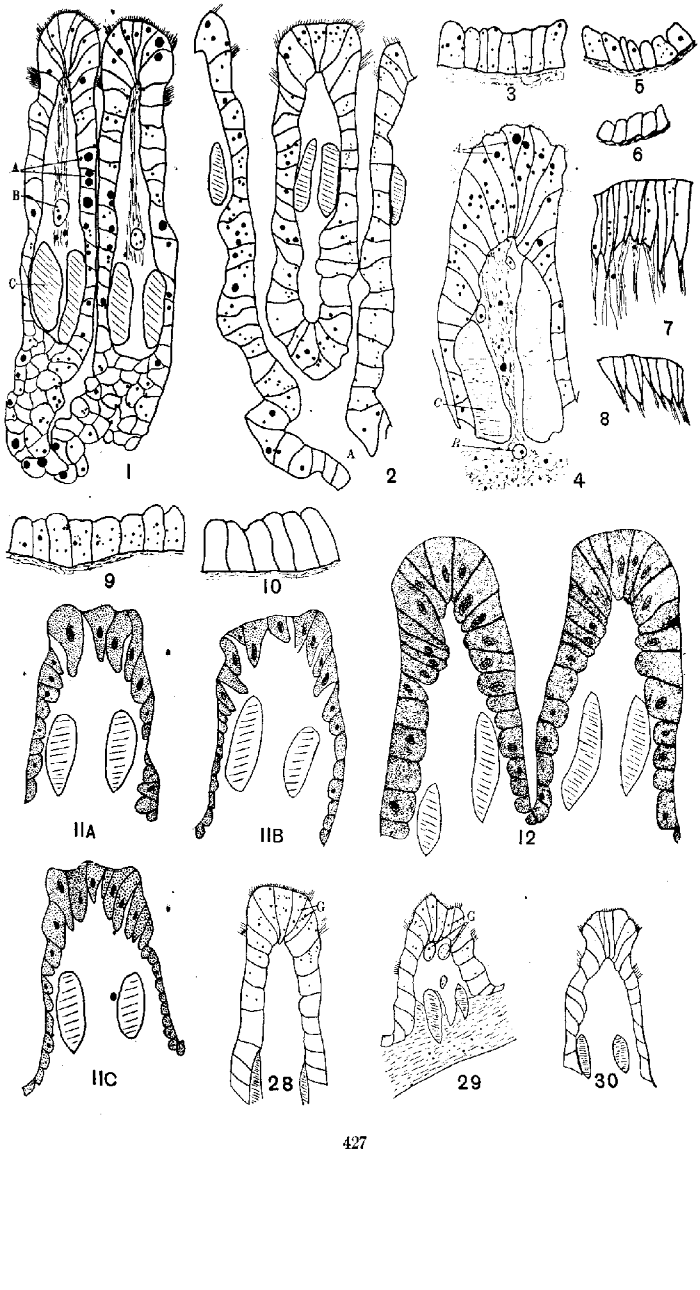
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PLATE 2

EXPLANATION OF FIGURES

13 Gill filaments of Q. ebenus which had been in filtered water for 58 days.

14 Gill filaments of Q. ebenus which had been in albumin solution for fifty-eight days. The relative depths of stippling in this figure and the preceding one have the same significance as in figures 11 and 12.

15 and 16 Gill filaments of Q. ebenus showing corpuscles entirely surrounded by epithelial cells. *D*, corpuscles.

17 and 18 Gill filaments of Q. ebenus which had been in albumin solution with mouth plugged for five days. The heavy dots represent the granules found in the cytoplasm and adhering to the outer ends of the cells. Apochromatic homoegeneous immersion objective and compensating ocular.

19 Gill filament of Q. ebenus used as control for those shown in figures 17 and 18. Magnification as in figures 17 and 18.

20 Gill filaments of Q. ebenus which had been for eight days in albumin solution stained with Janus green. *B*, green granules of albumin; *C*, corpuscles containing green granules.

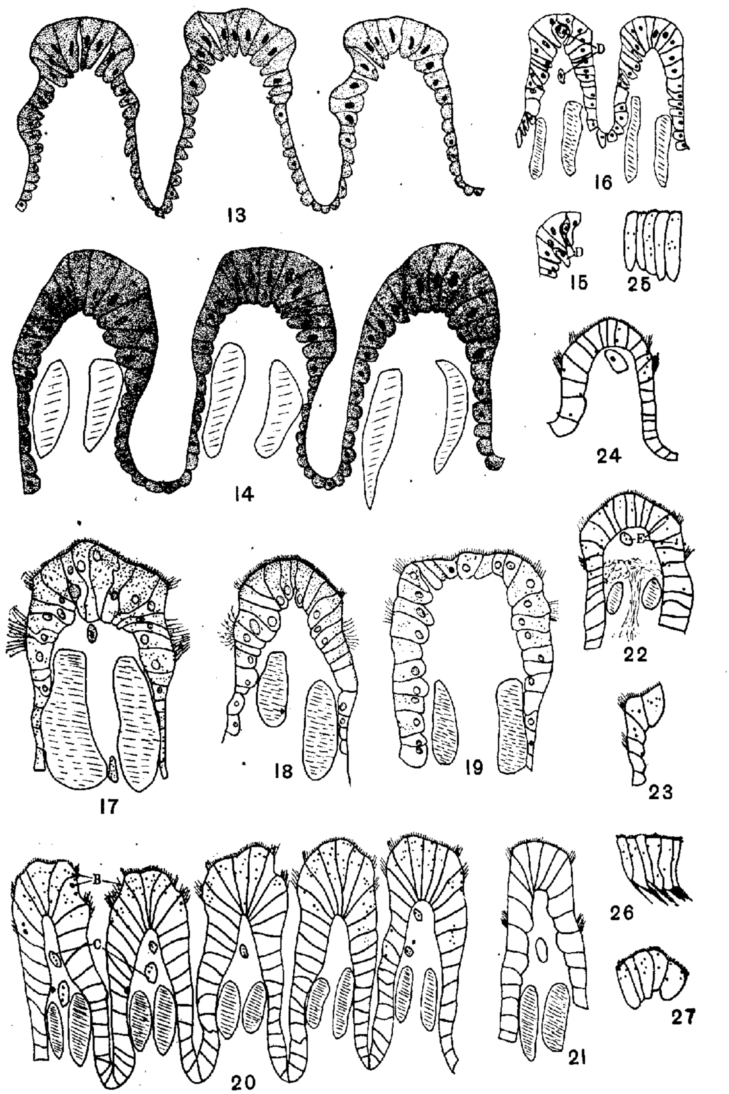
21 Gill filament of Q. ebenus which had been in water and Janus green for eight days. Control for figure 20.

22, 23 and 24 Gill filaments of Q. ebenus which had been with mouth plugged for eight days in albumin solution stained with Janus green. *E*, green granules.

25 Epithelial cells of palp of mussel shown in figures 22 to 24.

26 Epithelial cells of foot of mussel shown in figures 22 to 24.

27 Epithelial cells of mantle of mussel shown in figures 22 to 24. The black dots in figures 25 to 27 represent green granules of albumin.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THE MECHANISM OF ORIENTATION IN GONIUM

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TWO FIGURES

Recently Mast[1] has published a paper in which he tries to account for the mechanism of orientation in Gonium, using light as a directive stimulus. In 1911 Goodspeed and I[2] published an account of the orientation of the same form under the influence of a galvanic current. Mast does not seem to have been acquainted with our article since he makes no reference to it, although he confirms in the main, our observations.

In neglecting to make a study of the stroke of the individual flagellum, Mast has failed to consider an important factor and perhaps the chief factor in the orientation mechanism of this form. It seems necessary, therefore, to call attention to the analysis of orientation in Gonium which Goodspeed and I published five years ago. Since our paper appeared in a journal not readily accessible, I quote the section dealing directly with the subject in question.

In moving through the water the Gonium colony, in general, keeps its plane perpendicular to the line of direction. This may be modified by a more or less 'wobbly' motion. In addition to the progressive movement the colony rotates in its own plane. The direction of the rotation reverses frequently, seemingly without reference to the amount of linear motion. At times the rotation is suspended for a moment, while the organisms are moving forward.

With reference to the colony, the flagella extend forward and outward. In making a stroke occasionally the entire flagellum takes part, but usually only the peripheral one-half or one-third is used, while the inner part remains practically rigid. The stroke made by the active part of the flagellum describes a cone, the effective component of which is backward. There must be in addition a secondary effective component of the flagellar stroke in order to bring about the rotary motion.

If we observe the movement of an isolated cell it will be seen to describe a circle with the anterior end, in addition to moving forward. These two simultaneous movements cause the cell to follow a spiral path. If the cell reverses the direction of its progress it turns about in a wide half circle always keeping the flagella ahead. The turning may be accomplished by the flagella in one of two ways: 1) One fla-

[1] Mast, S. O., Jour. Exp. Zool. vol. 20, p. 1.

[2] Moore, A. R., and Goodspeed, T. H., Univ. Cali. Publ. Physiol. vol. 4, p. 17.

gellum remains inactive while the other continues the normal beat (fig. 1); 2) both flagella produce the effective component on the same side (fig. 2). Nothing of the nature of a motor reflex was observed.

Now Mast accounts for orientation to a directive stimulus by supposing the zooids farthest from the source of light to increase their activity and thus to bring the plane of the colony perpendicular to the lines of the stimulating force. He assumes equal activity of the two flagella in each cell of the colony, the behavior of the cells varying only in the intensity of their activity. He does not consider the possibility which Goodspeed and I pointed out; viz.: that the turning may be accomplished by an inequality in the beating of the two flagella of each cell. This we showed to take place in the isolated cells of Gonium. In case 1 the flagellum on the kathodal side ceases beating. This type of orientation was observed by Bancroft[3] in Volvox. In case 2 the flagellum on the kathodal side reverses the direction of its effective stroke. This mode of response was observed by Ludloff[4] in Paramecia.

The orientation of Gonium may therefore be accomplished either by increased activity of the cells away from the side stimulated, as Mast assumes, or by cessation of beat or reversal of stroke of the flagellum on the stimulated side of each cell. There remains the possibility that both types of reaction may play a part in orientation.

In view of the facts presented, it is clear that any thorough analysis of the phenomenon of orientation in Gonium must include not only a consideration of the changes in the activity of the cell as a whole but also of the differences in the activity of the individual flagella of each cell.

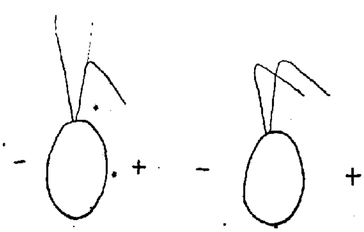

Fig. 1 Fig. 2

[3] Bancroft, F. W., Jour. Exp. Zool., vol. 4, p. 157.
[4] Ludloff, Archiv f. Ges. Physiol., vol. 59, p. 525.

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THE STRUCTURE OF METRIDIUM (ACTINOLOBA) MARGINATUM MILNE-EDWARDS WITH SPECIAL REFERENCE TO ITS NEURO-MUSCULAR MECHANISM

G. H. PARKER AND E. G. TITUS

SEVEN FIGURES (ONE PLATE)

ERRATA

THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, volume 21, number 3, October, 16. The chart on page 307 (E. R. Hoskins) is Chart 3 and belongs on page 1. The chart on page 311 is Chart 1 and belongs on page 307. The description of Chart 1 on page 307 refers to the chart on page 311, and the description Chart 3 on page 311 refers to the chart on page 307. This slip should be pasted on page 307 when the volume is bound.

In normally attached specimens the pedal disc is much extended, having a diameter decidedly greater than that of the column. It is marked by a system of somewhat irregular rays that indicate the lines of attachment for the mesenteries within. It often produces a dense brownish secretion that covers the surface to which the animal is attached and that is best seen in individuals that have been continuously resident on one spot for a long time.

The column is an approximately cylindrical portion which narrows from the expanded pedal disc to a region of more uni-

THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, VOL. 21, NO. 4
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form diameter, after which it expands as the oral disc is approached. It is ribbed longitudinally by the insertion lines of the numerous mesenteries. At about one fifth its length from the oral end, it is marked by a well defined circular collar indicating the position of the sphincter muscle. On the oral side of this collar the column wall is translucent and thin and the attachment of the mesenteries can be easily seen through it. On the pedal side of the collar, the column wall is much more dense and thick and the attachment of the mesenteries is indicated only by faint longitudinal ribs. This portion, moreover, is characteristically colored, brown, orange, whitish, or some mottled combination of these. This area when closely examined will be seen to present a considerable number of minute pores, the cinclides, by which the central cavity of the sea-anemone may communicate with the exterior. The finer structure of these openings has been well described by Carlgren ('93, p. 103).

The oral disc contains near its center the mouth, a more or less elongated opening surrounded by the protruding lips. The lips are coarsely grooved, the grooves extending downward into the oesophagus. Specialized lip grooves, the siphonoglyphs, are almost always present, commonly one, often two, and rarely three, a condition also found in M. dianthus by Carlgren ('93, p. 104). Surrounding the lips is a narrow intermediate zone carrying no specialized structures and connecting with the peripheral or tentacular zone. This is broadly lobed on the edge, where it passes over into the wall of the column; it carries a multitude of tentacles. The tentacles are largest near the mouth and become successively smaller as the periphery of the disc is approached. Their number is enormous and certainly amounts to thousands in a large individual.

We have been unable to confirm Carlgren's statement ('93, p. 104) that the tentacles of Metridium have terminal pores. We could not identify these pores in sections nor by forcing fluid through them. If a Metridium is anæsthetized in a solution of magnesium sulphate in sea-water, it is then easy to inject into its gastrovascular cavity dissolved methylene blue.

After the color has made its way into the cavities of the tentacles, they may be tied off one by one at the base and put under pressure till they burst. In no case did the colored fluid issue from the tip of the tentacle; but invariably from an obvious rupture on the side and we, therefore, feel quite convinced that Thorell ('59, p. 15) was correct in declaring the tentacle of Metridium to be without terminal pores.

The mouth leads into a long oesophagus which reaches well down into the gastrovascular cavity. The siphonoglyphs, one, two, or three, as highly differentiated grooves extend from the lips inward over the whole length of the oesophagus and terminate at the inner opening of this organ. The coarse grooves of the lips are continued as a system of finer corrugations inward over the whole length of the oesophagus.

The mesenteries extend into the gastrovascular cavity in pairs from their attachment on the inner face of the column wall and serve to unite the oral and pedal discs. Certain of these, the so-called incomplete mesenteries, extend only a short distance into the gastrovascular space where they terminate with a free edge. Others, the complete mesenteries, reach across the gastrovascular space and connect the column wall with the oesophagus. The complete mesenteries have a free edge only between the deep end of the oesophagus and the pedal disc.

A pair of complete mesenteries, the directives, connect each siphonoglyph, with the body wall. Each directive mesentery (fig. 1, compare fig. 3) carries on its free edge a mesenteric filament, which begins where the mesentery becomes free from the oesophagus and extends pedally over about half the edge of the mesentery till it merges into a much coiled organ which may be called the mesenteric convolution. At the pedal end of the convolution each mesentery gives rise to a single acontium. Orally the directive mesenteries are pierced by two apertures a large oral stoma (fig. 1, *stm.*) very near the lip and a smaller marginal stoma (*stm'.*) just inside the sphincter muscle (Hertwig, '79–80, p. 522; Carlgren, '93, p. 108). Each directive carries on its exocoel face a well differentiated longitudinal muscle,

which reaches from the oral to the pedal disc and lies close to the siphonoglyph. The directive mesenteries are always sterile.

Pairs of complete mesenteries, the non-directive, connect the column wall with the undifferentiated oesophagus, that is, with that portion which is not involved in the siphonoglyph. They reproduce (fig. 2) in general the structure of the directives, except that their longitudinal muscles are on their endocoel faces, are flatter, and lie rather in their middle portions than along their oesophageal borders. In some instances a non-directive mesentery may lack a marginal stoma or may have in place of it two or three small openings as though the original stoma had become partly divided (Carlgren, '93, p. 108).

The pairs of incomplete mesenteries range in size from those that are almost wide enough to be complete to those that are mere ridges on the inner face of the column. In the widest mesenteries (fig. 3) the highest degree of complexity is reached. Such a mesentery carries on much of the oral portion of its free edge a well differentiated mesenteric filament (*fil.*), below which is a mesenteric convolution (*cvl.*). From the lower limits of the convolution, an acontium (*ak.*) is given off. A series of gonads (*go.*) extends along the edge from a point about opposite the sphincter muscle to the region of the mesenteric convolution. The longitudinal muscle is well defined and on the endocoel face. There is naturally only one stoma, which corresponds to the marginal stoma of the complete mesentery (Hertwig, '79-80, p. 523; Carlgren, '93, p. 108). The smaller incomplete mesenteries differ from the one just described in the absence of certain parts. As one passes from the larger mesenteries with a full complement of parts to the smaller ones, the organs are omitted in the following sequence. The first part to disappear is the marginal stoma. Mesenteries that are so narrow as to be without a marginal stoma still possess gonads, filaments, convolutions, and acontia. The next organs to be lost are the gonads; all smaller mesenteries are sterile. Then follows the convolution. The simplest mesenteries are mere ridges on the column wall carrying small mesenteric filaments and

producing near their pedal ends small acontia. Finally at the angle of the oral disc and the column on the one hand and at that of the pedal disc and column on the other hand, pairs of small membranes appear which are evidently the beginnings of pairs of mesenteries not yet united over their middle portions. These incipient mesenteries sometimes show traces of filaments and acontia.

Aside from the fact that the mesenteries in Metridium are almost invariably disposed in pairs and are divisible into directives, non-directives, and incomplete mesenteries, there is very little about them that suggests the typical hexactinian. This is due to the great irregularity in their arrangement, a condition which has already been foreshadowed in the statement that Metridium is more generally monoglyphic than diglyphic and may even be triglyphic.

Moreover in diglyphic specimens the mesenteries are not necessarily arranged on the typical hexactinian plan. This matter has been much discussed, not only for Metridium (Parker, '97; McMurrich, '97; Torrey, '98; Parker, '99; Torrey, '02; Carlgren, '04; Hahn, '05; Carlgren, '09), but also for the closely allied genus Sagartia (Davenport, '03; Carlgren, '04; Torrey and Mery, '04; Carlgren, '09; Davis, '09), and the outcome seems to be that the irregularities in the number and arrangement of the siphonoglyphs and mesenteries in these forms are due to the prevalence among them of non-sexual methods of reproduction and, in the case of Metridium, particularly of basal fragmentation. But this question is not one of concern at the present, for, whether the siphonoglyphs and mesenteries in Metridium are regularly or irregularly arranged, the neuromuscular structure of this animal follows an essentially uniform plan.

II. THE MUSCLES

Thirteen fairly well-defined muscles or groups of muscles can be distinguished in Metridium. Many of them were long ago described and need only a word of comment, while others have not been recognized until recently.

1. The longitudinal muscle of the tentacle is an elongated conical sheet of muscle that forms the deepest layer of the ectoderm and is in direct contact with the supporting lamella of the tentacle. Its fibers course lengthwise in the grooves and on the crest-like elevations that extend up and down the outer surface of the lamella. They are more abundant and larger than the fibers of the circular muscle of the tentacle, but show no special grouping, being uniformly distributed around the whole tentacle (Hertwig, '79–80, p. 488).

2. The circular muscle of the tentacles is also an elongated conical sheet lying next the supporting lamella of that organ on its entodermic side (Hertwig, '79–80, p. 492). In this muscle the fibers take a circular course, and are fewer in number and finer than in the longitudinal muscle. They show no special differentiation except at the base of the tentacle, where there is a slight tendency to form a sphincter.

3. The radial muscle of the oral disc is made up of irregular dense bundles of ectodermic fibers more or less imbedded in the mesogloea of the disc. They radiate from the region of the mouth outward toward the periphery of the disc making their way between the bases of the tentacles (Hertwig, '79–80, p. 489).

4. The circular muscle of the oral disc is a flat muscular ring, whose fibers take a course concentric with the mouth and are often much involved in the supporting lamella of the disc on its entodermic side. (Hertwig, '79–80, p. 495).

5. The circular muscle of the oesophagus is a cylindrical muscle ensheathing the oesophagus on its entodermic face (Hertwig, '79–80, p. 517). It is not very strongly developed and its fibers, which take a circular course, are more or less interrupted where the complete mesenteries are attached to the oesophageal wall.

6. The circular muscle of the pedal disc is a system of fibers concentric with the disc and more or less imbedded as circular bundles in the inner face of the supporting lamella of the disc. This muscle is a well developed, vigorous organ (Carlgren, '93, p. 105).

7. The basilar muscles are radial strands which extend along the mesenteries at the junction of these organs with the pedal disc. There is a pair of these muscles for each mesentery and they vary in length in accordance with the size of the mesentery to which they are attached. On the larger mesenteries each muscle reaches from the periphery of the disc to about its center; on the small mesenteries from the periphery only a short way toward the center. These muscles cross the fibers of the circular muscle of the pedal disc at right angles and lie only a very short distance orally from them. The morphological and systematic significance of the basilar muscles has been emphasized by Carlgren ('93, p. 107; '05).

The most complex group of muscles in Metridium is that found on the mesenteries: it consists of the longitudinal, the transverse, and the parietal muscles.

8. The longitudinal muscles of the mesenteries (figs. 1, 2, 3, *mu. lg.*) are sheets of muscle fibers on the exocoel faces of the directives, on the endocoel faces of the non-directives and of most of the incomplete mesenteries. These muscles extend from the oral to the pedal disc.

On a directive mesentery the longitudinal muscle (fig. 1) is thickened into a very marked longitudinal ridge, which in the middle of its course runs close to the siphonoglyph and nearly parallel with it. Orally this thickened portion turns somewhat away from the chief axis of the animal and ends near the bases of the tentacles; aborally it runs nearly parallel with the free edge of the mesentery spreading out somewhat fan-like as it approaches its termination near the central portion of the pedal disc. Toward the oesophagus from this ridge the longitudinal muscle is represented by a very thin layer of longitudinal fibers. In the opposite direction the ridge gradually grows thinner and thinner till finally it, too, becomes a very thin sheet of longitudinal fibers.

On the non-directive and larger incomplete mesenteries (figs. 2, 3) the longitudinal muscle (*mu. lg.*) shows a band-like thickening in place of the ridge just described. This band takes much the same course on these mesenteries that the ridge does

on the directives; it is continued peripherally, and in the complete mesenteries centrally, into thin sheets of longitudinal fibers which cover the rest of the mesentery. The longitudinal muscles are absent from the very small incomplete mesenteries.

9. The transverse muscles of the mesenteries (figs. 1, 2, *mu. t.*) are very thin sheets that cover the endocoel faces of the directives and the exocoel faces of the other larger mesenteries; that is, they are on faces opposite to those on which the longitudinal muscles are located (Hertwig, '79–80, pp. 527, 530). They are not only very thin but they are very uniform layers of fibers, whose direction is approximately transverse to the chief axis of the animal. Commonly, however, they slope a little orally as they take their course from the periphery toward the center of the animal. They are better developed on the complete mesenteries than on the incomplete ones, from the smaller of which they may be entirely absent.

10. The parietal muscles of the mesenteries consist of longitudinal ridges on the exocoel and endocoel faces of almost all mesenteries at their line of junction with the column wall. On the larger mesenteries these muscles are small and inconspicuous in comparison with the other musculature of these organs, but on the very small mesenteries they are almost if not quite the only muscles present. We have not attempted to distinguish here what Carlgren ('05) would probably call the true parietal muscle, namely, that on the endocoel side of a non-directive mesentery, from what may be the remains of the parietobasilar muscle on the exocoel side of the same organ, for the reason that the parietobasilar may be entirely absent in Metridium. The muscles on the two sides of the mesenteries in Metridium are so strikingly similar that it seems to us more probable that we are dealing with a pair of parietals, the parietobasilar being absent (Hertwig, '79–80, p. 527, Taf. 17, fig. 1; McMurrich, '01, p. 8), than that this muscle is present in a reduced condition (Carlgren, '93, p. 107).

11. The circular muscle of the column is a cylindrical sheet of fibers covering the entodermic face of the supporting lamella of the column from its attachment to the pedal disc to the

region of its transition to the oral disc. The fibers in their circular course pass the lines of attachment for the mesenteries at right angles but are not to any great extent interrupted at these lines. The circular muscle is a well developed sheet (Hertwig, '79–80, p. 502).

12. The sphincter (fig. 2, *spht.*) near the oral end of Metridium is a firm circular band of muscle embedded in the supporting lamella of the column at the level where the thick, colored portion of this structure passes into the thin, more translucent part. Although the bundles of fibers that make up the sphincter are fully embedded in the supporting lamella, their distribution shows at once that they have come from the entodermic side of the body wall and that the sphincter is to be regarded merely as a differentiated part of the circular muscle of the column (Hertwig, '79–80, p. 505; Carlgren, '93, p. 105).

In small specimens the sphincter contracts in such a way that the oral aperture of the actinian is reduced to a minute pore. In large specimens this aperture under full contraction is more frequently an elongated slit. The length of the sphincter under the conditions of relaxation and contraction is very different; in a large specimen the relaxed sphincter measured 37.5 cm. in length and contracted 4.2 cm., or about one-ninth its former length.

13. The longitudinal muscle of the acontium in Metridium is best demonstrated in a transverse section of this thread-like organ. In such a section the axial region of the acontium is seen to be occupied by a mass of supporting lamella roughly T-shaped in outline and so placed that the shaft of the T points away from the center of the acontium. The longitudinal muscle consists of two bands of delicate fibers one on each side of the shaft of the T and away from the face of the acontium on which the nematocysts are situated.

These observations agree with the statement made by Carlgren ('93, p. 135) for the acontia of Metridium dianthus, but are opposed to those made by the Hertwigs ('79–80, p. 564) for the acontia of the closely allied genus Sagartia. According to the Hertwigs the longitudinal muscle band is to be seen on the outer

face of the cross arm of the T instead of on the sides of its shaft, probably a mistake in observation, as pointed out by Carlgren ('93).

III. NERVOUS STRUCTURE

Modern conceptions concerning the nervous system of the coelenterates and especially of the actinians date from the publication of the well known researches by the Hertwigs in 1879 and 1880. According to these investigations the epithelial covering of the actinians, to turn at once to that group, contains many sense cells whose deep ends branch often and form thus a nervous meshwork of the finest fibrils resembling the punctate substance described long ago by Leydig as one of the constituents of the central nervous organs in invertebrates. The nervous fibrils originating from the sense cells are supplemented by others from certain, large, deep-seated cells, the so-called ganglion cells, and it is through this combination of fibrils that the still deeper layer of muscle fibers is brought into action. This type of nervous structure according to the Hertwigs, pervades much of the body of the actinian, entoderm as well as ectoderm. It is stated by them to be best developed in the ectoderm of the oral disc and to form there a primitive central nervous organ from which connections pass to the tentacles, oesophagus, etc. On the column wall and foot this nervous mechanism is said to be much reduced. The ectodermic constituent is believed to be completely separated by the supporting lamella from the entodermic part except at the inner margin of the oesophagus where ectoderm and entoderm are continuous. Here the union is supposed to be especially mediated by the mesenteric filaments, by means of which nervous impulses are believed to reach the mesenteric muscles and the acontia (Hertwig, '79–80, p. 49).

Almost exactly this view of the organization of the actinian nervous system has been recently expressed by Wolff ('04, p. 274).

Grošelj ('09, p. 302), who has studied the nervous composition of the sea-anemones by means of methylen blue, also claims that the nervous system is distinctly centralized about the oral

pole, but maintains that the ectoderm of the oesophagus, not that of the oral disc, is the region of chief concentration. For him the oesophagus is the central nervous organ of the actinian.

In opposition to the view that the actinian nervous system is centralized is that which holds it to be diffuse. This view is the natural outcome of the earlier studies by Nagel ('92), Loeb ('95), Parker ('96), and others and has been supported more recently by the work of Bethe ('03) and especially of Jordan ('08, '12). The same view has also been urged by Havet ('01), whose histological studies have led him to conclude that the nervous elements in Metridium are not sufficiently concentrated to justify the expression centralised. They are diffusely scattered.

But Havet ('01, p. 411) has not only declared for a diffuse nervous system in actinians; he has also claimed grounds for changing in certain important details the scheme of nervous interaction proposed by the Hertwigs. According to Havet the so-called ganglion cells described by the Hertwigs, are really motor nerve-cells which receive impulses from the sense cells and transmit them to the muscles. Thus the actinian nervous system, according to Havet, contains in miniature the essential sequence of cells as found in an organ like the vertebrate spinal cord. Havet also claimed to have demonstrated a much closer relation between the ectodermic and entodermic nervous layers than was suspected by the Hertwigs. According to him ('01, p. 400) nervous fibrils can be shown by means of the Golgi method to pass from the ectoderm through the supporting lamella to the muscles of the entoderm and thus establish a direct union between structures that, according to the Hertwigs, were only indirectly united through the oesophagus. Moreover, the supporting lamella in Metridium is said to contain ganglion cells (Havet, '01, p. 395).

These ideas revive in a way the opinion early advanced by von Heider ('77, '95), that the supporting lamella in Sagartia, Zoanthus, and other actinians contains nervous elements, a claim that has been supported by the work of Hickson ('95, p. 371), of Ashworth ('99, p. 277) and of Kükenthal und Broch ('11,

p. 528) on the alcyonarians, though the assertions of the first two authors on this point have been called in question by Wolff ('04, p. 257) and by Kassianow ('08 a, '08 b). From a physiological standpoint Carpenter ('10, p. 159) has pointed out for certain corals the probability of just such nervous connections as have been described by Havet.

In attempting an elucidation of the nervous system of Metridium, a variety of methods were used. The isolation method, as employed by the Hertwigs, yielded important confirmatory results. Serial sections subjected to a variety of stains were also found serviceable. By the Golgi method many of the results obtained by Havet were confirmed. Material was prepared by the methylen-blue method, by the Bielschowsky, the Ranson, and the Ramon y Cajal methods, but only after an essentially new procedure was devised were results of importance obtained. This method was worked out by Dr. E. G. Titus and is briefly as follows:

Small specimens of Metridium, a centimeter or less in diameter, were taken directly from the sea and rinsed for a few seconds in distilled water, after which they were immersed for ten minutes in a 5 per cent solution of silver nitrate. The silver-nitrate solution with the abundant precipitate was then poured off and the specimen was again rinsed in distilled water after which it was put in a fresh 5 per cent silver-nitrate solution for half an hour during which the solution was once changed. It was then passed quickly through graded alcohols, 30, 50, 70, and 90 per cent, and left for about an hour in absolute alcohol. Next it was carried through a reversed series of graded alcohols to distilled water in which it was washed an hour by agitation and with frequent changes. From the water it was transferred to a 5 per cent solution of silver nitrate in which it remained five days. Then it was rinsed in distilled water and put in a hydrochinon solution containing 2 grains of hydrochinon in each 100 cubic centimeters of a 5 per cent solution of formaldehyde in water. Here it remained 30 hours after which it was rinsed in water, dehydrated in alcohol, passed through xylol to paraffine and cut into paraffine sections 10 to 15 micra thick.

The sections were mounted in xylol balsam under a cover glass and have proved permanent. The method is best carried out with the least exposure of the material to light. It is also well to use cold fluids, about the temperature of melting ice, up to the change to silver solution for five days. This and the subsequent changes to the paraffine were carried out at room temperatures. As with most metal impregnations, the results of this method are precarious, but in good preparations, the neurofibrils appear as black or brownish-black lines in an almost transparent field. In studying preparations one precaution is necessary. The impregnation stains the filaments of the large nettle capsules in the acontia very deeply and in many preparations these are found to have been discharged and to have entered such parts as the column wall and the mesenteries. Care must therefore be taken not to confuse them with nerve fibrils; but a few moment's inspection is usually enough to make sure what a given fibril is.

The ectoderm of the tentacles in Metridium exhibits a neuromuscular structure much like that ascribed to actinians in general by the Hertwigs. The epithelial sense-cells with their basal ends branching into fine fibrillae, can be demonstrated by the isolation method, the Golgi method, and that described in this paper. In the actinians studied by Grošelj ('09) these cells were often stained by methylen blue. In Metridium the nervous layer to which the central processes of these sense-cells give rise, is of considerable thickness and is directly next the longitudinal muscle fibers. According to the Hertwigs ('79–80, p. 488) this layer in the tentacles of many actinians contains at most only a few scattered ganglion cells and in the forms studied by Grošelj ('09, p. 294) none whatever were found. In the nervous layer of the tentacular ectoderm of Metridium, Havet ('01, pp. 408, 411) was unable to identify ganglion cells at all and we, too, have looked there in vain for these structures. We have examined for this purpose certainly many thousand sections of these organs but without being able to discover a single well defined ganglion cell. Nowhere have we seen anything comparable to the cells shown by Schneider ('02, p. 623,

fig. 510) in the tentacles of Anemonia. If such cells do occur in the ectodermic nerve layer of Metridium, they are certainly extremely rare; in the vast majority of cases therefore the basal branches from the sense cells must connect directly with muscle fibers, that is, without the intervention of ganglion cells. Havet's scheme of nervous connections, sense-cell to ganglion cell and ganglion cell to muscle cell, assuredly does not hold for the tentacular ectoderm of Metridium. Here the organization is obviously a simpler one, sense cells through their basal fibrillae connecting with muscle cells.

In sections of the tentacles of Metridium, in which the nervous layer in the ectoderm is easily demonstrated, it was impossible to be certain of such a layer in the entoderm. This holds true irrespective of the direction in which the tentacle is cut. The limits of the entodermic epithelium of the tentacle are well defined. Next its free edge the cells are filled with densely staining protoplasm, but as the base is approached they become more open in structure and their termination is in conjunction with the thin sheet of circular muscle fibers without the intervention of a nervous layer. According to the Hertwigs ('79–80, p. 493, Taf. 18, fig. 6) the nervous layer in the tentacular entoderm of Tealia occurs some distance from the circular muscle; Schneider ('02, p. 623) describes this layer in Anemonia directly in contact with the muscle, and a similar situation is claimed for it by Wolff ('04, p. 249) in Heliactis.

Havet ('01, p. 410) makes no mention of a nervous layer in the tentacular entoderm of Metridium dianthus and states that in this situation sense cells are extremely rare. In Metridium marginatum, as already stated, we have been unable to identify any entodermic nervous layer whatsoever and we have therefore been led to doubt if such is really present.

Another portion of the body of Metridium whose nervous structure is important is the column wall. The ectoderm of the column wall in actinians is said by the Hertwigs ('79–80, p. 500) and most recent investigators (Schneider, '02, p. 627) to contain only an insignificant amount of nervous tissue and yet scarcely any portion of the body of Metridium is more sen-

sitive to stimulation than this very part. A slight touch with a delicate glass rod or the discharge of a small amount of dilute acid on the lower portion of the column in Metridium is very sure to be followed by a vigorous contraction of the longitudinal muscles of the mesenteries and a consequent withdrawal of the oral disc. Our own observations on the nervous contents of the column wall as seen in sections agree very well with what has been described by the Hertwigs. The ectoderm of this wall, unlike that of the tentacles, exhibits neither a nervous layer nor a muscular layer. Nevertheless by isolation methods sense cells can be shown to be present and their connections can be inferred from an experiment like the following.

A fairly large area of the column wall of Metridium can be isolated from the rest by passing an incision around it in circular form. Such a plate of tissue, which in large animals may have a diameter of several centimeters, when thus cut, remains organically attached to the animal only through its mesenteries, and yet when it is touched by a glass rod or stimulated by weak acid, the whole animal responds by a normal contraction. This response fails when the mesenteries of the partly severed piece are completely cut and the piece is allowed simply to lie in place but without organic connection with the rest of the animal. We therefore believe that in Metridium there are nervous connections from the sense cells of the ectoderm directly through the supporting lamella into the mesenteries, as claimed by Havet ('01, p. 400).

In our own preparations we have found by the method described in this paper that the supporting lamella of the column wall contains many fibrils which course around the animal more or less horizontally (figs. 5, 6). They have been seen occasionally to enter the base of the ectoderm, but they are as a rule limited to the supporting lamella and the deeper part of the entoderm. In this last position they have already been identified by Wolff ('04, p. 251) in Heliactis. Where, in Metridium, the mesenteries arise from the column wall, these fibrils often branch and many of them pass out into the mesogloea of the mesenteries (fig. 5). Their course here is approximately radial but after

they reach the band of longitudinal muscle, they often extend up and down this band in intimate relations with its fibers (fig. 7). Many of the fibrils that enter the muscle terminate there in small knobs (compare fig. 7), which may be a simple form of motor ending comparable to that which Wolff ('04, p. 249) has described in Heliactis and Grošelj ('09, p. 287) in Bunodes; or these endings may be only apparent and mark the point at which the impregnation ceased, not the real end of the fibril.

We regard these fibrils as neurofibrils and as the principal part of the transmission system between the ectoderm of the column wall and the longitudinal muscles of the mesenteries. None of our preparations show these fibrils connected with cells. It would seem that the method we have used stains only the neurofibrillar substance and not the rest of the generating cell. Whether the cells that produce these neuro-fibrils are those long since known to be in the supporting lamella (fig. 4) and easily demonstrated by ordinary stains, or whether they are cells in the ectoderm, we are unable to say. It seems to us not improbable that some of the cell bodies identified by Havet ('01, p. 395) in the mesogloeal regions of the body wall in Golgi preparations may be those from which the neurofibrils have come, but we agree with Kassianow ('08 b, p. 672) in the belief that at least some of the bodies figured by Havet as ganglion cells are artifacts, though we do not go so far as to raise the question whether he saw any ganglion cells at all. But however this may be, we are confident that the fibrils that we have seen are an important part of the nervous connections between the ectoderm of the column on the one hand and the longitudinal muscles of the mesenteries on the other, and that these connections are in the main through the supporting lamella. Our results are thus opposed to the conclusion drawn by the Hertwigs ('79–80, p. 50) namely, that the ectodermic nervous system of actinians is in connection with the entodermic only through the œsophagus, and confirm the observations of Havet to the effect that there are mesogloeal connections between these two systems, as was long ago maintained by von Heider

for actinians and more recently by Hickson, Ashworth, and Kükenthal und Broch for alcyonarians.

Since from any point on the ectoderm of the lower part of the column the longitudinal muscles of all the mesenteries can be brought into action, it follows that the neuromuscular mechanism of the column and adjacent parts must be much more extensive and complex than that of the tentacles. Besides sense cells and muscle cells there are very probably in the parts under consideration intermediate cells which, if not motor cells in the sense of Havet, are at least transmitting cells connecting the sensory mechanism with the motor. If this view is correct the ectodermic sense cells are only indirectly connected with the mesenteric muscles and a system more complex than that in the tentacular ectoderm must be present.

When a spot on the lower portion of the column in Metridium is stimulated, the response that follows, as already stated, is in the longitudinal muscles of the mesenteries. The circular muscle of the column, though nearer the point of stimulation than the mesenteric muscles, exhibits no obvious activity. Probably its tonus is increased by the contraction of the longitudinal muscles, but of this we have no evidence. If the stimulated spot on the column is now covered with crystals of magnesium sulphate, these will dissolve and in a few minutes the whole area will be found to be fully anesthetised. The spot may now be freely touched without calling forth a contraction of the longitudinal muscles. As a result of such stimulation, however, a horizontal band of contraction soon appears and passes slowly round the column. This constriction is undoubtedly due to the contraction of certain of the circular-muscle fibers in the body wall and since the nervous mechanism of this region has been rendered inoperative by anesthetisation, these fibers must have been stimulated directly. We therefore conclude that while the column wall has nervous connections by which the longitudinal muscles may be brought into action, it also contains muscle fibers that contract in response to direct stimulation. The normal stimulus for these muscles has not been definitely determined, but it is probably the stretching to which they are

subjected by the changes in pressure within the animal. The neuromuscular mechanism of the column wall in Metridium consists, then, not only of a nervous arrangement by which distant muscles can be called into operation but also of a system of muscle fibers acting under direct stimulation. Whether the circular muscle is at all under the influence of nerves cannot be definitely stated, but certain peristaltic movements that it shows in recently fed individuals (Müllegger, '13) suggest that it is under more or less nervous control.

The pedal disc of Metridium shows many of the same neuromuscular peculiarities that the column wall does, but we have not had the opportunity to work out its organization to the same extent as we have that of the wall. The ectoderm of the disc is thicker than that in the column and is more richly provided with gland cells. Its supporting lamella contains a system of neurofibrils exactly like those in the column wall. These fibrils take in general a circular course concentric with the center of the disc and can be traced in among the circular-muscle fibers, the basilar muscles, and even into the bases of the mesenteries themselves. The cellular relations of these fibrils are as obscure as are those of the fibrils in the column wall. Whether these fibrils control the musculature of the disc or of the mesenteries or both was not determined. Their presence as mesogloeal elements is the chief fact that we have to contribute to the anatomy of this part of the actinian.

In the oral disc, the lips, and the oesophagus we were unable to obtain good impregnations, and we got evidence of only a few circular neurofibrils in the supporting lamella of the oral disc. The nervous layer of the tentacular ectoderm can easily be traced in ordinary preparations into that of the ectoderm of the oral disc, from which it can be followed over the lips and into the ectoderm of the oesophagus. This is the region that has been regarded by the Hertwigs ('79–80, p. 50) as centralized in function and the layer which can be traced into the oesophagus is the beginning of the connection with the entodermic nervous system. That the ectoderm of the lips and the oesophagus in Metridium exhibits such a layer cannot for a moment be

doubted, but that this layer has the great nervous importance attributed to it by the Hertwigs is by no means so certain. Contrary to the statement made by Nagel ('92), the lips of actinians are sensitive to many forms of stimulation, but that they and the adjacent parts of the oesophagus form an important organ of nervous transmission may be fairly questioned. If a Metridium is cut in two except for the lips and more or less of the oral part of the oesophagus, nervous transmission is seriously interfered with. The stimulation of one half of such an animal is followed by a contraction in that half alone, though if only a small bridge of the column wall is left connecting the two pieces transmission between them is easily accomplished. We, therefore, are led to conclude that notwithstanding the fact that the lips and oesophagus contain nervous elements intimately associated with the adjacent parts, they are not organs of special nervous transmission as maintained by the Hertwigs and certain others workers.

Another organ whose neuromuscular structure is of interest is the acontium. In Metridium a single actontium is attached to the free edge of each mesentery not far from its lower limit. These organs, as already stated, have a mesogloeal axis which in transverse section is roughly T-shaped. The muscle of the acontium is in the form of a series of fibers closely applied to the two sides of the stem of the T.

We agree with Carlgren ('93, p. 94, 135) in being unable to confirm the statements made by the Hertwigs ('79–80, pp. 562–565) as to the neuromuscular structure of the acontium of Sagartia. In their figure the muscular layer is on the opposite side of the mesogloea from that on which Carlgren finds it in Sagartia, and both he and we find it in Metridium. The rather diagramatic figure of a transverse section of an acontium from Metridium dianthus given by Havet ('01, p. 406, pl. 5, fig. 37) is so sketchy as to make its interpretation difficult. This author, however, claims that both sense cells and muscle fibers are present in this structure. Just external to each row of muscle fibers is a thin layer of punctate substance which has been generally regarded as nervous (compare Hertwig, '79–80, p. 564,

Taf. 21, fig. 11). Structurally the acontium would seem to be an ideal organ in which to test the transmission of nervous impulses and the like, through its primitive nervous strands. Pieces of acontia four or five centimeters long can be easily obtained from a large Metridium and will continue alive and active in seawater for many hours. When such a filament is mechanically stimulated by agitating it in seawater or by dropping seawater on it, or when it is flooded with dilute meat juice, it twists itself into an irregular coil. This response takes place slowly and only after a minute or two. If the stimulus is limited to one end of a long acontium that end and that end only responds by becoming snarled. This reaction will occur as well at the central end as at the peripheral end of a given acontium. When acontia have been kept for twenty minutes or so in seawater containing chlorotone, a period long enough to anesthetize the tentacles of an intact Metridium, they will still become snarled when flooded with dilute meat juice exactly as unanesthetized acontia do. When acontia still attached to a Metridium but extending several centimeters away from it are variously stimulated at their free ends, not the least response has ever been observed in the Metridium itself though the acontia react vigorously in the region to which the stimulus is applied. The stimulation of their free ends seems to have no more influence on the Metridium than the cutting of the free end of a long hair has on a human being. From these observations we conclude that the acontia in Metridium have no nervous significance whatever and that their muscles, are stimulated directly and in no other way. What the so-called nervous tissue of the acontium really is we cannot say. Possibly, as one of us has already suggested (Parker, '12, p. 461), it may be a system of more open spaces next the muscle cells and so arranged as to facilitate their nourishment and the removal of their waste. Of this, however, we have no proof. What we wish to emphasise is the completely non-nervous nature of the acontium.

IV. DISCUSSION

The neuromuscular mechanism in Metridium when studied in detail presents much greater variety than previous investigations have described. There is no uniform plan of organization in these important structures that applies generally to the whole animal but a variety of conditions obtains. At least four types of organization occur. First there are muscles whose action appears to be quite independent of nervous control such as the longitudinal muscles of the acontia. These muscles are extremely slow in their responses; their contractions are known to follow a mechanical stimulation only after an interval of a minute or so, and their activity continues even after the tissue in which they are imbedded has been permeated with an anesthetic. These muscles may be said to represent the group of independent effectors, such as are seen in the muscles of sponges, in the embryonic vertebrate heart, and so forth.

A second type of neuromuscular organization is seen in the circular muscles of the column wall and possibly also of the tentacles. These, like the acontial muscles, are normally open to direct stimulation but are probably also somewhat under nervous control.

A third type of neuromuscular organization is seen in the tentacular ectoderm of Metridium. Here peripheral sense cells are closely associated with muscle fibers. This system acts with relatively great rapidity, a contraction following a stimulation in less than a second. It also exhibits nervous conduction. Complete anesthetization abolishes its activity and leaves the muscle incapable of responding to ordinary stimuli. In its essentials it may be said to consist of receptors combined more or less immediately with effectors.

The fourth type of neuromuscular organization in Metridium is such as is seen in the system including the sense cells of the column wall, the longitudinal muscles of the mesenteries and the connecting tracts. This type brings into physiological relation distantly located parts. Like the third type its activities are quickly carried out and are also easily obliterated by anesthetics.

It includes not only receptors and effectors but probably also intermediate elements in the form of a nerve net through which conduction is accomplished and in which it would not be surprising to find the beginnings of central functions.

The four types of organization thus briefly described are probably not simply types of structure which meet special requirements in the sea-anemone's activities, but they have, we believe, a certain phylogenetic significance. The first, the independent effector, represents the initial step in the evolution of the neuromuscular mechanism as realized in the sponges (Parker, '10). The second and third, the combined receptor and effector, introduce the first nervous element into this series whereby greater efficiency in the discharge of the motor mechanism is attained. And the fourth marks the first appearance of that intermediate structure which in its incipiency is merely a connecting and transmitting organ from receptor to effector but which in its final outcome is the central nervous apparatus of the higher animals. Thus the diversity of neuromuscular organization in Metridium includes non-nervous as well as nervous muscular response (Parker, '12) and seems to us to have a certain significance for the phylogeny of the nervous system.

V. SUMMARY

1. The muscular system of Metridium consists of thirteen fairly well defined muscles or classes of muscles.

2. These muscles represent at least four types of organization: first, independent effectors, as seen in the longitudinal muscles of the acontia; secondly, simple receptor-effector systems, like the circular muscles of the column, which, though brought into action by direct stimulation, are probably also under some nervous control; thirdly, more highly specialized receptor-effector systems like the longitudinal muscles of the tentacles, which respond only through nervous stimulation; and fourthly, complex receptor-effector systems as shown in the sense cells of the column wall in conjunction with the longitudinal muscles of the mesenteries.

3. These four types of organization may be regarded as representing developmental steps in the evolution of the neuromuscular mechanism of the higher animals.

4. The nervous system is not limited to the ectoderm and to the entoderm but in certain regions it penetrates the supporting lamella and thus connects one layer of the body with the other; the supporting lamella thus comes to contain nervous elements.

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PLATE 1

EXPLANATION OF FIGURES

All figures are from Metridium (Actinoloba) marginatum Milne-Edwards. Figures 1 to 3 are semidiagrams; figures 4 to 7 are camera drawings.

ABBREVIATIONS

ak., acontium
cvl., mesenteric convolution
ec'drm., ectoderm
en'drm., entoderm
fil., mesenteric filament
go., gonad
lab. lip
ms'enr., mesentery
ms'gl., mesoglea
mu.lg., longitudinal muscle
mu.t., transverse muscle
n'fbrl., neurofibril
spht., sphincter
stm., oral stoma
stm'., marginal stoma

1 to 3 Semidiagrams of mesenteries as seen in dissected specimens that had been anesthetized with magnesium sulphate and hardened in 5 per cent formaldehyde in sea-water. In each drawing the oral end of the mesentery is uppermost and the oesophageal edge is toward the left. Natural size.

1 A directive mesentery.

2 A non-directive, complete mesentery; the marginal stoma, usually single, is represented by two holes.

3 An incomplete mesentery showing the maximum number of organs present on such mesenteries.

4 Column wall seen in horizontal section; stained with Mayer's acid carmine; the mesoglea, the only part in which the detail is drawn, contains many cells as indicated by the numerous nuclei. Magnification 100 diameters.

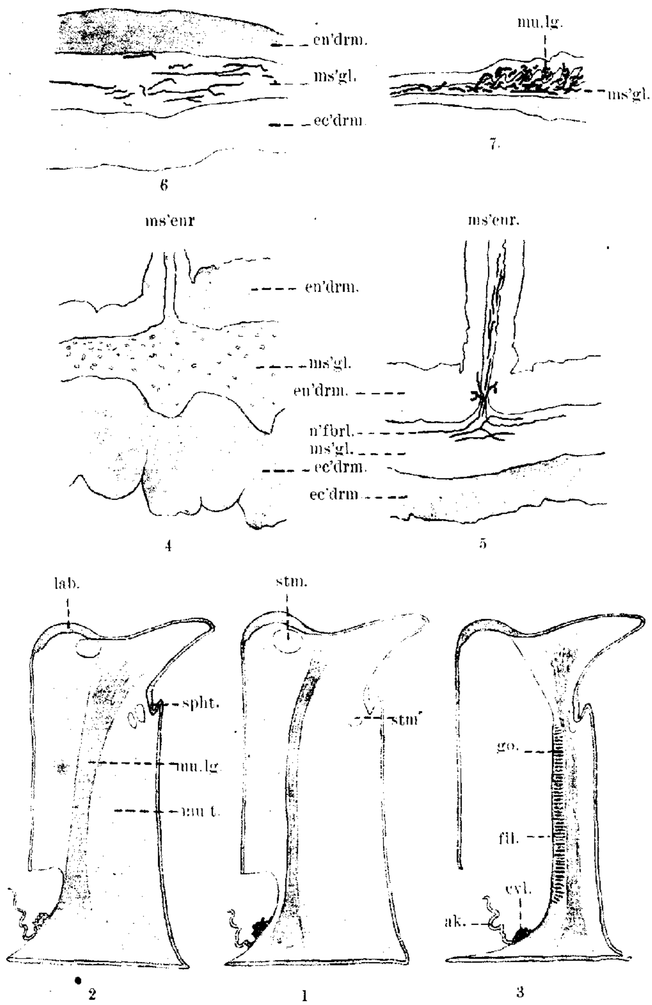
5 to 7 Drawings from three preparations showing neurofibrils stained by the Titus method; magnification 100 diameters.

5 Column wall seen in horizontal section; the neurofibrils in the mesoglea of the column are seen passing into the mesogleal axis of a mesentery; the neurofibrils show branching and, at the base of the mesenteric mesoglea, some fibrils penetrate the entoderm in the region of the parietal muscles.

6 Column wall seen in horizontal section; branching neurofibrils occur in the mesoglea.

7 An incomplete mesentery seen in horizontal section in the region of its longitudinal muscle; its mesoglea contains many neurofibrils which enter the entoderm where the longitudinal muscle-fibers are located; some of the neurofibrils apparently terminate here in small knobs.

G. H. PARKER AND E. G. TITUS



CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 282

THE EFFECTOR SYSTEMS OF ACTINIANS

G. H. PARKER

Every animal possesses means, more or less well defined, for responding to environmental changes and in many groups of animals these means are differentiated into definite systems of organs, the effector systems. In any given species the effectors may be independent of, or more or less under the control of, the nervous system. They are obviously often very diverse. In the actinians the most conspicuous of these systems are the mucous system, the nematocyst system, the ciliary system, and the muscular system. With each one of these the animal is capable of responding to particular elements in the environment and all have been suspected of being in one way or another under nervous influence. The nature of the systems and their relations to the nervous mechanism in the actinians will be taken up in the following pages.

THE MUCOUS SYSTEM

Every part of Metridium seems to be able to produce mucus, and, under normal conditions, all the surfaces of this animal are covered with a thin layer of slimy secretion. This is produced in all probability by the so-called mucous cells and albumen cells which have been described by Schneider ('02) in Anemonia as occurring throughout the ectoderm especially in the oesophagus and the mesenteric filaments, and generally, though less abundantly, throughout the entoderm.

If an expanded Metridium is submerged in fresh water for about fifteen seconds and then returned to sea-water, its whole outer surface and especially that of its tentacles becomes covered with a profuse secretion of mucus thus showing the extreme ease with which this material is produced. When a Metridium has been kept in an aquarium whose water supply is not of the

best, the animal commonly envelops itself in a mucous coat, which is often sloughed and forms a loose tube about its base. This secretion of mucus, which occurs in response to a great variety of stimuli, mostly noxious, is undoubtedly a means of protection, as has been suggested by Duerden ('06) and by Gee ('13), and recalls the more permanent secretions of such actinians as the cereanthids, in which protective tubes are regularly produced.

But mucus is not only discharged in response to noxious stimuli; it is also produced through the action of favorable influences. Meat juices call forth, especially from the tentacles, a profuse discharge of mucus which renders these organs extremely sticky. In this way the capture of food is greatly facilitated. After Metridium has been submerged in fresh water for a brief period the mucus discharged on its oral disc is not usually simply sloughed off but much of it may be swallowed as it is in such corals as Favia and Fungia where, according to Duerden ('06), the chief means of feeding is by swallowing the mucus of the oral disc in which small organic fragments, etc., have been entangled. This method of feeding is not known among the actinians, but the response of Metridium just described shows how easily the actinian method of capturing food could be converted into that employed by Favia and Fungia.

If a given spot on the outer surface of the column of Metridium is touched three or four times with the end of a small, blunt glass-rod, a slight clot of milky mucus will soon appear. This clot is to be seen in the exact region of stimulation and though this region often shows a considerable depression due to muscular contraction which may spread horizontally half way round the column, the secretion of mucus always remains strictly localized and gives no evidence of being extended through muscle contraction or nerve transmission.

Dilute acids and dilute alkalies also bring about a free discharge of mucus. Juice from the meat of the killifish, Fundulus, when applied to the isolated tentacles or acontia likewise produces a free discharge of mucus. The discharge of mucus on the acontia forms a tube through which this organ gradually makes its way by ciliary action.

Wolff ('04, p. 250) has described nerve fibers in connection with the gland cells in actinians and von Uexküll ('09 a) has expressed the opinion that the secretion of mucus in these animals is under the control of their nervous mechanism. The fact that on stimulation mucus is discharged only over the surface stimulated and never beyond this area gives no support to this view but suggests rather that this activity is due to local stimulation unassisted by nervous action. This opinion is rendered probable by experiments made on anaesthetized actinians. If a Metridium that has been shown to secrete mucus on mechanical or on stimulation by weak acid is put in seawater to which magnesium sulphate has been added, the animal will cease to respond through its neuromuscular mechanism in about ten minutes. Nevertheless it will discharge mucus freely but locally to appropriate mechanical and chemical stimulation and this power will remain in full vigor even after the animal has been in the magnesium solution seven hours. Hence it would appear that the secretion of mucus is not dependent upon nerves.

Similar results were obtained from chloretone. After a submersion of ten minutes in seawater containing chloretone, Metridium ceased to respond through its neuromuscular mechanism to mechanical stimulation. Notwithstanding, it secreted mucus to appropriate local stimuli, and this recurred even after 6 hours of immersion in the chloretone seawater. From the observations already recorded and from the results of the experiments on anaesthetization, I believe we are warranted in concluding that the nervous system of actinians is not involved in calling forth the secretion of mucus but that this activity is accomplished by the direct stimulation of a body of independent effectors, the specific gland cells of the ectoderm and entoderm.

THE NEMATOCYST SYSTEM

No cellular constituents are more characteristic of any large group of animals than the nematocysts are of the cœlenterates, for the occurrence of these organoids in certain mollusks seems to be due to a process of digestive appropriation from cœlenterate sources. The offensive and defensive character of nematocysts

has long been recognized and much has been written about their method of development and the means by which they are exploded. It is not my intention to take up a discussion of these problems but to limit myself to the single question of the relation of the nematocyst to the nervous mechanism of sea-anemones as seen in normal activity.

In 1871 Schulze showed that when the nematocysts in Cordylophora were discharged by impact with a foreign body this discharge took place only at the spot where the foreign body came in contact with the animal. Schulze showed further that the nematocyst cells were provided with a small bristle-like structure, the cnidocil, which projected beyond the general surface of the animal and served as a trigger for the explosion of the nematocyst itself. Some eight years later the Hertwigs ('79-80) discovered branched basal processes on the cells which produce the nematocysts and believed these to be nervous in function. Thus it came to be assumed that under appropriate nervous stimulation large numbers of nematocysts could be discharged in moments of need. This theory of the nervous discharge of the nematocysts was supported from one standpoint or another by von Lendenfeld ('83), Chun ('91), Schneider ('02), Wolff ('04), Grošelj ('09) and Baglioni ('13), while other investigators were rather inclined to accept some of the various modifications of the theory of non-nervous discharge as originally advanced by Schulze. Metridium offers some excellent opportunities for testing these two general views.

Several types of nematocysts occur quite commonly in the ectoderm of Metridium. Small cysts with short slender filaments occur sparingly on the mesenteric convolutions and in the ectoderm of the column wall. Large ones are found scattered over the oesophagus. But the regions in which the nematocysts are especially developed are the tentacles and the acontia. On stimulating the tentacles with dilute hydrochloric acid, great numbers of rod-like bodies about 20μ in length together with many fine filaments at least 140μ long are discharged. On similar treatment of the acontia, a perfect forest of nettle filaments are discharged. These come from capsules about 45μ

in length and consist of a spirally marked, basal stalk some 70μ long and a terminal filament of over 700μ length, the total extent of these elements being at least twenty-five times that of the shortest nettling organs in this animal.

To ascertain the relation of the nematocysts to the nervous system in actinians the following experiments were carried out on the tentacles and the acontia of Metridium. A normal acontium can be cut from an animal and placed under a microscope without bringing about the discharge of its nematocysts. If, now, it is flooded with carmine in seawater or with a solution of methylene blue in seawater, its cilia can be seen to strike toward what was its distal end, but no nematocysts will be exploded. If, next, it is flooded with HCl $\frac{n}{2}$, a profuse discharge of nematocysts occurs. Judging from the observations of Glaser and Sparrow ('09) probably most acids would cause this reaction. Distilled water will also bring about the explosion of the nematocysts, but as a rule only a few are thus discharged, for, if the treatment with water is followed with dilute acid, a renewed discharge immediately takes place. Some samples of methyl green have been found to produce a very complete explosion of the nematocysts but others have not, a fact which indicates that the discharge was probably produced by some associated impurity rather than by the methyl green itself. A few nematocysts are always discharged near the cut end of the acontium, and if an acontium is shot in and out a pipette in seawater, many of the nematocysts will be found discharged. These organoids, however, are not exploded when the acontium is flooded with juice from the flesh of a fish (Fundulus) though they do discharge in fair numbers when a small piece of this flesh is brought into contact with them. This reaction is probably dependent upon a mechanical rather than a chemical stimulus from the flesh.

The tentacles of Metridium discharge their nematocysts to dilute acids, certain samples of methyl green, mechanical insult, and more or less to distilled water but not to carmine nor methylen blue nor to meat juice in seawater. In fact the nematocysts of the tentacles respond to the various stimuli I have tried in precisely the same way as those of the acontia do, and I have

been unable to find even a slight difference between these two sets of nematocysts such as might be inferred to exist from the table given by Glaser and Sparrow ('09, p. 367).

When the portion of the tentacle or acontium that receives an effective stimulus is compared with that from which the nematocysts are discharged a striking condition is found. With mechanical stimulation the nematocysts are discharged only in the immediate region of the application of the stimulus, as observed long ago in Cordylophora by Schulze ('71); with chemical stimulation apparently the same is true. If the distal end of a fragment of an acontium or a tentacle is treated with dilute acid, the nematocysts are discharged at that end and nowhere else. If the proximal end is similarly treated, they explode only in that region. There is thus no evidence in these two organs of nervous transmission in either direction so far as the nematocysts are concerned. But the relation of the stimulated area to the area of discharge is best seen when the stimulus used is a colored fluid such as methyl green. If a small crystal of that kind of methyl green which will cause the discharge of the nematocysts is brought near a living acontium, the nematocysts can be seen to explode as they become covered by the diffusing green solution and as they are never discharged in advance of the cloud of colored fluid, the evidence for the local action of the stimulus uninfluenced by transmission, is very conclusive.

These observations are in exact agreement with those of Wagner ('05, p. 618) on Hydra and I therefore conclude that nematocysts are discharged by a local stimulus and not by an impulse that has been transmitted from a distance. Is this local action, however, of a direct kind or does it involve a minutely circumscribed nervous mechanism? Such a question is not to be answered by localized stimulation but must be approached by other means. Two drugs are known which completely abolish nervous activity in many lower animals, including the actinians, and which therefore may be used in testing this matter. They are chloretone and magnesium sulphate.

If a large Metridium is put in seawater containing magnesium sulphate, in a very short time it becomes insensitive to stimulation and will remain so for a long time, recovering only when it is transferred to pure seawater. If from such an anæsthetized animal a few tentacles are cut and these are flooded with a dilute solution of hydrochloric acid in seawater, they will discharge their nematocysts precisely as normal tentacles do. Exactly similar results can be obtained from the acontia. So far as the explosion of the nematocysts is concerned, I have been unable to distinguish between an acontium that has been anæsthetized with magnesium sulphate and a normal one. The same is true of acontia that have been treated with chloretone. After a prolonged immersion in a solution of chloretone in seawater, acontia and tentacles in which there is every reason to believe that the nervous activity is completely abolished, will still discharge their nematocysts on appropriate stimulation precisely as the unanæsthetized parts do. It therefore seems clear that even the circumscribed local response which has been shown to be characteristic for nematocysts is not dependent upon any influence that may be rightly called nervous, but is determined by a direct stimulation of these elements as was implied long ago by Schultze ('71). If the nematocysts contain myofibrils, as was first maintained by Chun ('81) for Physalia and has since been claimed by Jickeli ('83), Schneider ('90) Will ('09) and others, the activity of these elements must depend, as Will has pointed out, rather upon some form of direct stimulation, than any thing that can reasonably be called nervous.

From the preceding discussion it seems fair to conclude that the basal processes of nematocyst cells assumed by the Hertwigs to be nervous in function are in reality not so and that these elements are without nervous connections, as, in fact, Hadzi ('09) has recently claimed for them in Hydra. There seem to be, therefore, no grounds, either anatomical or physiological, for the assumption that the nematocysts are effector endorgans of the nervous system and, of course, no grounds for the view held by von Lendenfeld ('83, p. 369) that they are under the control of the animal's will. My opinion is in entire accord

with that of Wagner ('05) that nematocysts are effector organs exploded by direct stimulation and not under the control of a nervous mechanism. This view makes clear how these organoids may be appropriated by another animal, such as a nudibranch mollusk, and still retain their effectiveness, a condition which would be difficult to explain if this effectiveness was in any sense dependent upon nervous action.

THE CILIARY SYSTEM

There seems to be ample ground for assuming that large ciliary organs, such as the swimming plates of the ctenophores, are under the influence of the nervous system of the animals in which they occur (Parker, '05 c; Bauer, '10), and it is therefore natural to raise the question whether any of the cilia in Metridium are thus controlled. The cilia in this and other actinians have been more fully studied than any other of the effector systems possessed by these animals and the results of such investigation show in general a striking agreement. The pedal disc of Metridium is without cilia. The same is true of the column wall in this form (Parker '96, p. 109; Carlgren, '05, p. 311) as well as in Halcampa and Sagartia (Carlgren, '05), though in Protanthea and Gonactinia it is said to be more or less ciliated (Carlgren, '05). In Metridium (Parker, '96, p. 112; Carlgren '05, p. 311), Sagartia (Vignon, '01, p. 475) and Halcampa (Carlgren, '05, p. 311) the cilia of the oral disc beat toward the periphery of that part, while in Protanthea and Gonactinia (Carlgren, '05, p. 310) their effective stroke is toward the mouth. In all actinians with ciliated tentacles thus far observed the ciliary currents are from the base to the tip of these organs (Metridium, Parker '96, p. 110; Allabach, '05, p. 37; Carlgren, '05, p. 311; Sagartia, Vignon, '01, p. 475; Carlgren, '05, p. 311; Aiptasia, Jennings, '05, p. 453; Protanthea, Gonactinia and Halcampa, Carlgren, '05, pp. 310, 311). The siphonoglyphs in Metridium, whether one, two, or three, possess cilia that invariably sweep inward (Parker, '96, p. 113; Carlgren, '05, p. 312) as do also those in Sagartia (Vignon, '01, p. 475; Carlgren, '05, p. 312). This fact was long ago pointed out for the alcyo-

narians by Hickson ('83, p. 694) who observed that in these animals the current in the single siphonoglyph was inward whereas that in the rest of the mouth was outward.

The cilia on the non-siphonoglyphic portion of the lips and œsophagus in the great majority of actinians and even in some corals, ordinarily beat outward, and they may be brought by appropriate stimulation to reverse temporarily and beat inward (Metridium, Parker, '96, p. 109, 1905 a, 1905 b; Carlgren, '05, p. 312; Allabach, '05, p. 37; Sagartia, Vignon, '01, p. 475, Torrey, '04, p. 212; Tealia and Actinostola, Carlgren, '05, p. 318; Cribrina, Gee, '13, p. 324; and in such corals as Fungia and Favia, Duerden, '06, pp. 596, 604, and Isophyllia, Carpenter, '10, p.153). This reversal is accomplished by the chemical and mechanical stimulation of food materials. In my first study of this subject I was led to conclude that in Metridium marginatum ciliary reversal could not be accomplished by mechanical stimulation, as has been demonstrated by Torrey ('04) in Sagartia davisi, but subsequent work has convinced me that in specimens of Metridium that have been a week or so without food, this reversal, as suggested by Jennings ('05), can be accomplished by mechanical as well as by chemical means, thus confirming Allabach's statement for this species ('05, p. 37). The continuous inward current mentioned by Carlgren ('05, p. 314) as occurring in Sagartia viduata was seen in animals that had been kept a week without food and was probably in reality a temporary reversal of an unfed animal to mechanical stimulation, for ciliary reversal has already been observed in other species of Sagartia (S. parasitica, Vignon, '01, p. 475; S. davisi, Torrey, '04, p. 212). Whether the inward current in Gonactinia and Protandra (Carlgren, '05, p. 310) is of this nature or not, cannot be stated with certainty, but in view of the facts just presented, it seems possible.

The cilia on the mesenteric filaments in Metridium marginatum have an effective stroke from the pedal toward the oral end of these organs and, as Torrey ('04, p. 214) states, are not known to be subject to reversal. The cilia of the mesenteric convulutions are very active and irreversible, but because of the great

irregularity of the courses of the convolutions I was unable to determine the sense of the direction in their sweep. On the acontia the cilia beat persistently toward the free end of these organs, as already described by Torrey ('04, p. 214).

Excepting for the matter of reversal, there is nothing in the action of the cilia of Metridium, or in fact of those of other actinians, that would lead to the suspicion that these organoids are under nervous influence; and in those that reverse their effective stroke the reversal is so strictly local in reference to the stimulus that no nervous interpretation of this special activity is suggested (Parker, '96, p. 114). The same strict agreement in the localization of stimulus and area of reversal has been pointed out by Duerden ('06, p. 596) for the coral Fungia, indicating that in this group also the ciliary reversal is probably non-nervous.

Not only does agreement in the distribution of stimulus and of response favor a non-nervous interpretation of ciliary action in actinians, but experiments with anæsthetics also support this view. If a Metridium whose labial cilia have been shown to reverse to meat, is placed quickly in a large volume of sea-water containing some chloretone and allowed to remain there for five minutes, all traces of neuromuscular activity will disappear, though ciliary reversal will still occur with great precision. The reversal also takes place in a perfectly clear and indisputable manner in animals that have been anæsthetized with magnesium sulphate. Since these two substances completely abolish neuromuscular activity and yet interfere in no essential way with the ciliary reversal and other like activities in Metridium, it is safe to conclude that the cilia of this and other actinians, unlike the swimming plates in ctenophores, are quite independent of nervous control and in this respect are like the cilia in higher animals. In this particular, then, the ciliary system in Metridium is like the mucous system, and the nematocyst system in this animal, an effector mechanism independent of nervous control.

THE MUSCULAR SYSTEM

The muscular system in Metridium consists of at least thirteen muscles or groups of muscles (Parker and Titus, '16) which show a rather unusual degree of differentiation when it is recalled that this animal is one of the lower metazoans. Notwithstanding this specialization, Metridium, like most other actinians, goes into a state of such complete contraction when vigorously stimulated and remains for so long a time entirely closed that the general impression made by it is that it is a simple muscular sac capable of only one general form of response. This condition is both misleading and instructive.

The prolonged contraction of Metridium shows that one of the most characteristic features of actinian muscle, like that of many other invertebrates, is its very high degree of tonicity as compared with the corresponding tissue of vertebrates. This contrast has been emphasized of late by von Uexküll ('09 b) and especially by Jordan ('07, '08, '12).

This exhibition of excessive tonicity, however, has led investigators away from a finer scrutinizing of the muscular responses in actinians, responses which will be found to justify, I believe, the high degree of anatomical differentiation already pointed out. It is from this standpoint that I wish to give an account of some of the muscular reactions of Metridium.

The longitudinal and parietal muscles of the mesenteries. These muscles extend lengthwise of the mesenteries and for the most part from the pedal disc to the oral disc. By their action the oral disc with its crown of tentacles is drawn down toward the attached pedal disc (Hertwig, '79, p. 527). In the non-directive mesenteries, the longitudinal muscles are on the endocoel faces of these organs, on which they show as a thickened median band which spreads out as one approaches the oral or the pedal disc. The arrangement and concentration of the fibers in these muscles is especially favorable for a depression of the oral disc. On the directive mesenteries the longitudinal muscles are on the exocoel faces and each muscle is thickened as a longitudinal ridge close to the accompanying siphonoglyph. As the wall

of the siphonoglyph in Metridium is firm, almost as stiff as a thin layer of cartilage, it is quite clear that these muscles are so concentrated as to exert a tension favorable to folding the siphonoglyph walls when the animal contracts. The parietal muscles extend up and down the length of both sides of the mesenteries next the column wall. This, like the wall of the siphonoglyph, is somewhat resistant to folding and the parietals are undoubtedly especially concerned with drawing it together in general contraction. Thus the longitudinal muscles and the parietals show a certain amount of specialization adapted to their particular tasks and yet constitute a single physiological system for the depression of the oral disc.

The means by which these muscles can be brought into action are extremely diverse. If a Metridium is kept in running seawater in the dark, it soon attains to its fullest expansion. Under such circumstances its height may be six times the diameter of its column. If now it is suddenly illuminated by diffuse daylight or a strong electric light, it will gradually shorten its column to about one third or one fourth its former length, but without contracting its oral disc. This operation is accomplished by the simultaneous and united action of the longitudinal and parietal muscles and, so far as can be seen, necessitates the coöperation of no other muscles. In this change of form some of the water in the gastrovascular cavity is discharged through the mouth; in consequence of the retention of the rest the diameter of the animal is increased, thereby putting the circular muscle of the column under a certain tension. Recovery from this state is more gradual than its assumption and is dependent partly on ciliary action whereby water is returned from the outside through the siphonoglyphs to the gastrovascular cavity, and partly by the action of the circular muscle of the column, which by pressing on the fluid contents of the gastrovascular cavity thus pushes the oral disc away from the pedal disc. The return to the expanded state is doubtless also in part dependent upon the relaxation of the longitudinal and parietal muscles, a condition which comes about when the animal is in the dark in running seawater. Whether there is any reciprocal relation of a more

intimate kind than has been suggested (compare Sherrington, '06) between the longitudinal mesenteric muscles and their opponent, the circular muscle of the column, I do not know; nor can it be stated whether or not the longitudinals work against other muscles such as the transverse muscles of the mesenteries.

There are many other ways beside changes in illumination by which the contraction of the longitudinal muscles of the mesenteries may be brought about. Thus a mechanical stimulation of the tentacles or of the column wall or a chemical irritation of these parts is almost always followed by a sudden and vigorous contraction of the longitudinals, but these forms of stimulation also bring into action the sphincter and the muscles of the oral disc and the column, and thus carry the action far beyond that of a simple muscle response. So far as I am aware, a change in illumination is the only means whereby the longitudinal and parietal muscles of the mesenteries can be brought into action unassociated, as far as can be seen, with other muscles.

Changes in illumination, moreover, are the only means which I have found to call forth a partial activity of the longitudinal and parietal muscles. In a general illumination these muscles contract uniformly and the oral disc, retaining its horizontal position, slowly descends. If, instead of illuminating a sea-anemone generally, it is strongly illuminated from one side, it will contract much more rapidly on that side and come to rest with its oral disc turned toward the light, as already pointed out by Bohn ('06). This condition, which is the state of positive phototropism of a sessile animal or of a plant, has already been observed and photographed by Hess ('13, p. 436) in Cereanthus and Bunodes, and demonstrates the partial independence of the longitudinals of one side from those of the other.

The sphincter. Many methods by which the longitudinal and parietal muscles of the mesenteries are excited to action also induce an ultimate activity of the sphincter whereby the oral disc after its withdrawal becomes covered by the upper part of the column wall. This is the usual final step in complete contraction, and it is natural to inquire whether the sphincter

can be excited to activity without being involved in a sequences of changes making up the total act of contraction.

If a number of specimens of Metridium attached normally to stones, pieces of shell, and so forth, are allowed to stand for a day or so in quiet seawater in which there is more or less decomposing material such as dead Mytilus edulis, many of the specimens will contract their sphincters even though their columns remain elongated. Though the longitudinal and parietal muscles of the mesenterics in these specimens may not be fully relaxed, they are nearly so. Certainly the only muscle in these animals which is in vigorous contraction is the sphincter, and this remains firmly and tightly closed until the animals are transferred to pure seawater. What it is in the foul water that stimulates the sphincter to independent action and what part of the body of the Metridium serves as a receptor for this stimulus, I have not been able to find out, but of the essentially isolated response of the sphincter under the circumstances mentioned there can be not the least question.

When a Metridium is placed in the dark in a strong flow of seawater, it usually expands to its fullest extent both in the spread of its oral disc and in the lengthening of its column. If now the flow of seawater is cut off, the animal is very likely to cover the oral disc by a contraction of the sphincter without shortening its column, at least to any great extent. Thus quiet seawater following current action induces an independent contraction of the sphincter much as foul seawater does.

The sphincter is opposed chiefly to the pressure of the fluids within the actinian's body and it is this probably that restores the sphincter on relaxation to its most expanded form. The internal pressure that is effective in this respect is due in part to the intake of water by ciliary means but particularly to the action of certain muscles such as the circular muscle of the column.

The longitudinal muscle of the acontium. When a Metridium has drawn down its oral disc and covered this region by the contraction of the sphincter, further stimulation is followed usually by the discharge of numerous acontia through the mouth and the cinclides. As many as seven of these thread-like bodies

may issue through a single cinclis. If the stimulus is unilateral, the acontia are discharged chiefly on the side stimulated, as Torrey ('04, p. 208) has already noticed in Sagartia. The acontia do not emerge in consequence of their own activity but are carried outward by the streams of water that are escaping under pressure through the mouth and the cinclides. After the acontia have emerged they are in no sense directed toward external objects, harmful or useful, but rest in long straightish lines on the surface of the actinian or they are wafted about slightly by the currents of water. They gradually disappear by being drawn back into the animal. They are ciliated and the effective stroke of their cilia is vigorous enough to move them bodily and is always toward their free ends, hence they themselves are moved by this stroke back toward their attachments. The discharge and the return of the acontia, therefore, are processes in which their contained muscle plays no part.

If an extended acontium is stimulated by having seawater vigorously squirted on it, in the course of one or two minutes it gradually draws itself up into a close snarl. If seawater containing a little meat juice is used instead of pure seawater, the snarl is more pronounced and remains for a longer time. Sooner or later the acontium untwists and straightens out preparatory to its withdrawal into the body of the actinian. The twisting and contorting of the acontium is brought about through the contractions of its longitudinal muscle the fibers of which, as already mentioned, are closely applied to the mesogleal axis of this organ. Evidence has already been advanced (Parker and Titus, '16, p. 451) to show that the acontial muscle is an independent effector and not under the influence of nerves and that no nervous transmission occurs through the acontia. The straightening out of the coiled acontium is due in part to its ciliary activity and in part probably to the elasticity of its mesogleal axis against which its muscle probably acts.

The longitudinal and circular muscles of the tentacles. In a resting, expanded Metridium the tentacles are usually quiescent, radially disposed, and directed in the main away from the mouth. If a tentacle in such an animal is touched or otherwise

mechanically stimulated, this organ responds by turning eventually toward the mouth of the animal, after which it gradually assumes its initial position. The movements thus induced are in the beginning without doubt due entirely to the activity of the longitudinal muscle but before long both longitudinal and circular muscles are certainly involved for, though there may be no nervous connection between them, their physical relations are so intimate that the activity of the longitudinal fibers may perfectly well serve as a mechanical stimulus to call into action the circular system. In this instance then, the longitudinal muscle of the tentacle may act independently in the beginning, but it is quickly followed by its natural opponent, the circular muscle.

If the tip of a tentacle in Metridium is cut off, the tentacle contracts, the wound almost at once closes with a nipple-like formation, and the tentacle with its tip firmly puckered gradually reexpands. In this condition it remains until the injured end is fully healed (Chester, '12). At the beginning of this operation, during which the tentacle is contracted, the longitudinal as well as the circular muscles are involved, but after the tentacle has reexpanded and before the wound has healed, a matter of a day or so, the circular fibers near the cut are the only muscular elements really active. Thus the circular muscle as well as the longitudinal may under particular circumstances give evidence of independent action.

The transverse muscles of the mesenteries. These muscles connect the column wall with the œsophagus. If a small amount of seawater is discharged into the mouth of an expanded, resting Metridium no response is usually noticeable. If the seawater is a $\frac{N}{20}$ hydrochloric acid solution, the actinian immediately opens the œsophagus widely and exhibits on its column a few well marked vertical grooves. These disappear gradually as the œsophagus closes. If the position of these grooves is carefully noted, it will be found that one is always present for each siphonoglyph and that the others are distributed in accordance with the arrangement of the other pairs of complete mesenteries. The grooves thus mark the lines of attachment of these mesen-

teries and are the result of the contraction of their transverse muscles which, are those concerned with the opening of the œsophagus.

If fragments of fish meat are put on the lips of a fully expanded Metridium, they are carried into the animal by ciliary action through an œsophagus which opens widely to receive them and during this operation the column of the animal is marked by the same vertical grooves that were seen in the experiment with acidulated seawater. As the pieces of food pass into the gastrovascular cavity the grooves fade out. It is clear, then, that the transverse muscles of the complete mesenteries are concerned with the expansion of the œsophagus for the reception of food.

If a piece of fish meat is placed upon the tentacles of an expanded Metridium, these organs become characteristically stimulated and if the meat is removed before it is brought by the tentacles to the animal's lips, the œsophagus will still open, accompanied by the formation of vertical grooves on the column. This response could not be elicited by the application of weak acid to the tentacles. Under such circumstances a withdrawal of the oral disc took place. Thus it appears that not every form of chemical stimulus that can be applied to the tentacles is followed by an opening of the œsophagus. To appropriate stimuli, however, the tentacles and lips may act as receptors for the opening of this tube. I have found no other parts of the body of Metridium from which I could elicit this œsophageal response.

The organ that would be regarded naturally as the opponent of the transverse muscles of the mesenteries is the circular muscle of the œsophagus. Its position is such that its contraction would bring about a closure of this tube and probably such is its action, though on this point I have no direct evidence. The internal pressure of the seawater contained in the animal, slight though it is, certainly aids materially in closing the œsophagus, as can be seen in a Metridium that is feeding. After the piece of food has passed down the œsophagus of such an animal, the walls of this organ held in the beginning more or less apart,

approximate gradually as though under slight pressure from the inside.

Transverse muscles are found not only on complete mesenteries but also on the incomplete ones, though in this situation they are rather poorly developed. Such muscles in consequence of their failure to reach the œsophagus can have nothing to do with its expansion. When a Metridium is fed, however, one can often see on its column beside the six or eight deep grooves marking the positions of the complete mesenteries, a whole series of minute grooves which, like the others, fade out as the food is swallowed. These are probably due to the transverse muscles of the incomplete mesenteries but their method of formation, and the significance of the contraction that produced them, if in fact it possesses any significance at all, have not been worked out.

The circular muscle of the column. This muscle is unquestionably the chief antagonist of the longitudinal muscles of the mesenteries and acts in conjunction with them in the contraction and expansion of the animal as a whole, but it also has its own activities. If a fully expanded Metridium is freely fed, it will usually show upon its column ring-like constrictions which form near the oral disc and proceed like a peristaltic wave over the column to the pedal disc. A new constriction appears every four or five minutes. These waves, which may have to do with the movement of the food within, have been noted by Gosse ('60, p. 253) in Halcampa, and very recently by Müllegger ('13, p. 487) in Metridium and Sagartia, in the latter of which they have been photographed. According to Müllegger they may run from the pedal to the oral pole as well as in the reverse direction. They represent without question a specialized and individual activity of the circular muscle, the receptor mechanism of which has not been ascertained.

Closely related to this peristalsis of the column are certain responses that can be induced in animals that are partly contracted but still well filled with seawater. If the column of such an animal is stimulated by rubbing it lightly on a particular spot with a blunt glass-rod, a constriction begins to form at the

spot stimulated and extends slowly around the column, an operation which is completed in about half a minute. If several spots at different heights on the column are similarly stimulated, as many as three such rings can be formed at once on the same individual. From the position and method of formation of these bands of constriction it is quite clear that they are due to the contraction of bundles of fibers in the circular muscle.

If in a completely contracted Metridium a spot on the exposed portion of the column is mechanically stimulated, a ring of constriction passes round the animal encircling the pore which marks the location of the mouth. If a radial cut is made from near the pore out to the periphery of such an animal and care is taken that this cut goes no deeper than through the column wall, the constriction resulting from a local stimulus on reaching this cut fails to pass across it, showing that this activity is entirely resident in the column wall. If now a fully expanded Metridium which will contract its tentacles when a given spot on the column wall is mechanically stimulated, has this spot fully anæsthetized by dropping on it crystals of magnesium sulphate, a ring of constriction will still form around its column when this spot is repeatedly touched by a blunt glass-rod. As the nervous mechanism in this portion of the animal has been rendered inoperative through the magnesium sulphate, it follows that the formation of the groove must be a purely muscular operation thus demonstrating independent action on the part of the circular muscle.

The radial and circular muscles of the oral disc as well as the basilar and circular muscles of the pedal disc form natural pairs of muscles that undoubtedly act in conjunction with each other in the movements of their respective parts. I have been unable, however, to find any means by which these muscles can be brought into action individually, though judging from the results obtained in other instances it would not be surprising if such means were sooner or later discovered.

This examination of the musculature of Metridium brings out very clearly the fact that its anatomical differentiation is not without physiological significance. Jordan ('08) has already

called attention to the striking contrast in the rate of action between the longitudinal mesenteric muscles in Metridium and such muscles as those of the sphincter or the foot in this form; the rate at which the animal can withdraw the oral disc is in the strongest contrast to that at which it covers the disc or creeps about. Jordan is inclined to ascribe this difference to the muscles themselves, but I have brought forward evidence to show that these differences in rate are dependent in part at least upon the presence or absence of nervous connections. The acontial muscles are extremely slow in response and the formation of a constriction groove by the circular muscle of the column is a matter of minutes. The first of these is, I believe, absolutely unassociated with nervous activity and the second can take place after nervous activity has been temporarily obliterated. It would therefore appear that these very sluggish movements are dependent upon muscle unassociated with nerve and that when these two elements are combined in a high degree of differentiation, as in the longitudinal mesenteric system, the rate of response becomes relatively high.

Another matter of general importance in the nervous activity of sea-anemones is the question of reflexes. It has been generally believed that the neuromuscular system of such an animal as Metridium could exhibit tonus and rhythmic motions, but nothing comparable to the sharply marked individual reflexes of the higher animals (Jordan, '08, p. 223), and this condition is in general true. But there are, nevertheless, some responses in Metridium that are strikingly like the individualized reflexes of the higher forms. For instance when acidulated sea-water is put upon the lips and the only response that follows is the opening of the œsophagus by the contraction of the transverse muscles of the complete mesenteries, a condition is presented which, since it can be revived again and again, shows all the characteristics of a highly individualized reflex. I am fully aware that this instance and others like it imply in the nervous structure of Metridium a definiteness of conduction tracts that argues against a diffuse nervous system, but I believe these cases to be exceptional and to represent merely the first signs

of that process by which out of an undifferentiated state the highly complex nervous organization of the higher animals has arisen. In my opinion there is not the least doubt that some of the neuromuscular responses in Metridium are true reflexes, though the majority of such operations are more usually exhibitions of excessive tonicity or of rhythmic motion.

SUMMARY

1. The effector systems of Metridium are at least four in number: the mucous, the nematocyst, the ciliary, and the muscular system.

2. The mucous, the nematocyst, and the ciliary systems are independent effectors and are not under the control of a nervous mechanism.

3. The muscular system, consisting of thirteen muscles or groups of muscles, shows a variety of conditions. Some muscles, such as the longitudinal muscles of the acontia, are independent effectors and are not under nervous control. Others, like the circular muscles of the column wall may act independently or under the influence of nerves. Still others, such as the longitudinal muscles of the mesenteries, act only in response to impulses from a relatively complex nervous mechanism.

4. Non-nervous muscular responses are carried out sluggishly and require a minute or more for completion. Nervous muscular responses are relatively rapid and may be accomplished in a second or so.

5. Notwithstanding that the whole musculature exhibits a high degree of tonicity, there are responses such as the expansion of the œsophagus by the action of the transverse muscles of the complete mesenteries which are of the nature of well individualized reflexes.

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EXPERIMENTAL STUDIES ON THE ORIGIN OF MONSTERS[1]

I. AN ETIOLOGY AND AN ANALYSIS OF THE MORPHOGENESIS OF MONSTERS[2]

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EIGHTY-NINE FIGURES

CONTENTS

[1] Aided by a grant from the Bache Fund of the National Academy of Sciences.

[2] Brief reports of the results of this work have been presented and demonstrations of material made at the meeting of the American Society of Zoologists in Philadelphia (December 29 and 30, 1914) and at the meeting of the American Association of Anatomists at New Haven (December 28-30, 1915). A preliminary communication was also published in the Anatomical Record (cf. Werber '15 c.)

I. INTRODUCTION

A review of the literature shows us that we are at the present time confronted with two theories regarding the causal genesis of monsters. The first and older one, the amniotic theory, is essentially a mechanical theory, while a more recent one which is based on recent experiments on the influence of chemical alterations of the egg's environment, might be spoken of as the chemical theory of teratogenesis.

The amniotic theory maintains that terata are due to anomalies of the amnion, which latter, by adhering too closely to the embryo or constricting it, is thought to bring about the various well known malformations. The inadequacy of this assumption has in recent years been repeatedly pointed out.[3] It is a well known fact that amniotic anomalies are found relatively rarely in malformed ova. In one hundred and sixty-nine pathological ova which Mall ('08) has examined he asserts that he has found not a single case of anomaly of the amnion. Wherever amniotic adhesions are found, Mall, I believe rightly regards them as incidental. It is not impossible that they may be syngenetic with some terata, i.e., due to the same causes which brought about the developmental deviation of the embryo. This might particularly apply to the experimental terata of Dareste ('91) and others, in which anomalies of the amnion were found. On the whole, it might well be said, that in view, particularly, of experimental results on anamniotes, the amniotic theory would hardly seem to deserve more than historical interest.

In rejecting this mechanical theory Mall traces monstrous development to faulty implantation of the ovum in a diseased uterus, which in turn makes adequate nutrition of the embryo

[3] Cf. Mall ('08) and Jordan ('09).

impossible. This is, essentially, a chemical theory of teratogenesis for it assumes the atypical development of the embryo to be due to lack of necessary substances.

More direct support for the chemical theory of teratogeny is offered by the recent investigations of Stockard ('07, '09, '10 a) and McClendon ('12 a and b), both of whom obtained one-eyed monsters by subjecting developing teleost eggs to the action of various toxic substances, such as magnesium chloride, alcohol, ether, alkaloids, etc. These investigations have shown that some such developmental deviations as are found to occur spontaneously may be brought about by the chemical action of various substances, and thus they suggest that atypical development in nature may be due to pathochemical alterations of the germ's environment.

The above considerations have led me to assume that in order to attack the problem of atypical development in nature effectively it is necessary to find the unusual chemical factors which cause the embryo in its natural environment to develop in a defective or monstrous manner.

Since the metabolism is the greatest source of chemical modifications of the body, I concluded that the solution of the problem of the causal genesis of monsters must be sought for in pathologic parental metabolism.

Starting from this assumption, I carried out, in the summer of 1914, some experiments on Fundulus heteroclitus, the fertilized eggs of which were subjected to the action of solutions of urea, butyric acid, lactic acid, acetone, sodium glycocholate and ammonium hydroxide. Conclusive results were so far obtained only with butyric acid and acetone. The (rather simple) methods employed have been described in a former paper (Werber '15 c) to which the reader is referred.

II. THE RECORDED TERATA

The results which were obtained are very much alike in both series of experiments, with butryic acid and acetone. The variety of deformities being almost endless in both, it would be practically impossible to present much more than certain types

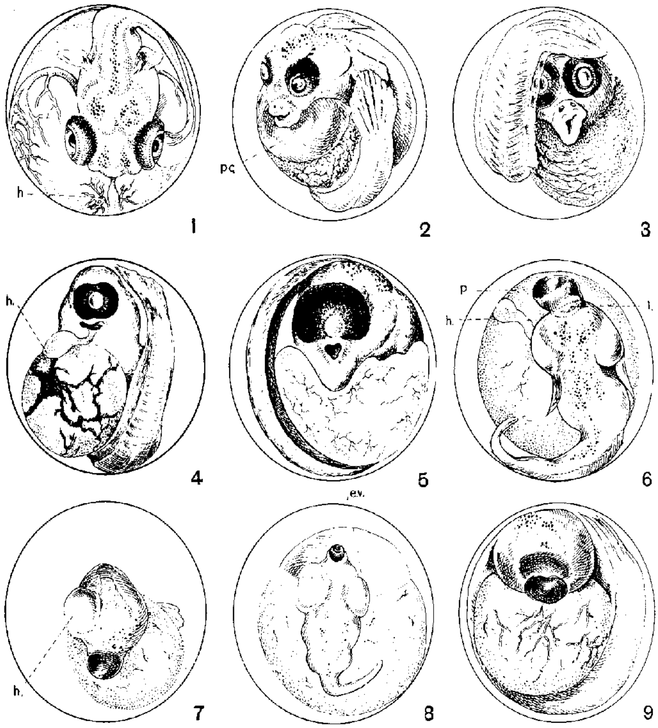


Fig. 1 Normal embryo of Fundulus heteroclitus, nine days old. *h.*, heart.

Fig. 2 Synophthalmia bilentica, from $\frac{1}{12}$ gram molecular butryric acid, twenty-eight days old. *pc.*, pericardial vesicle.

Fig. 3 Snyophthalmia bilentica, from $\frac{1}{12}$ gram molecular butyric acid solution, twenty-eight days old.

Fig. 4 Synophthalmia unilentica, from $\frac{1}{12}$ gram molec. butyric acid, twenty-four days old.

Fig. 5 Cyclopia synophthalmica, from acetone solution (35 cc. to 50 cc. sea-water) twenty-six days old.

Fig. 6 Cyclopia perfecta, from $\frac{1}{12}$ gram molec. butyric acid, twenty-four days old. *l.*, lens; *p.*, pigment epithelium; *h.*, heart.

Fig. 7 Cyclopia perfecta, from acetone solution (35 cc. gram molec. to 50 cc. sea-water) twenty-five days old.

Fig. 8 Cyclopia perfecta, from $\frac{1}{16}$ gram molec. butyric acid, thirteen days old. *e.v.*, ear vesicles.

Fig. 9 Cyclopia perfecta, from $\frac{1}{12}$ gram molec. butyric acid, twenty-eight days old.

of them. In a previous publication (Werber '15 c), I have presented a brief survey of the recorded terata. While a more complete presentation of them is attempted in this paper, I am fully convinced that it is inadequate to convey the proper impression of the great range of variation in the noted effects.

1. Terata of the head

The malformations of this part of the body are many and unusually varied. Indeed, as will be pointed out later, this part of the body appears to be the most susceptible one to the influence of toxic solutions. The deformities of the head affect the sense organs, the brain, the mouth, and the skull. All of them are usually found to occur in various combinations. The most striking ones on examination in toto are those that concern the eyes.

a. The ophthalmic terata. When Fundulus eggs are subjected to the action of butyric acid or acetone in the concentrations stated above, embryos with normal eyes are of the rarest occurrence. Cyclopia, i.e. the presence of a single median eye is found very often. I have likewise recorded in my observations a wide range of intermediate stages between two normal eyes in the typical position in the head all the way down through more or less closely approximated eyes or eyes of an apparent double composition and true cyclopia to complete anophthalmia as described so often by the older teratologists and as obtained experimentally in recent years by Spemann ('04), Lewis ('09), Stockard ('09, '10 b), and others.

A comparison of figures with the normal embryo in figure 1 is very instructive of this gradual transition from the normal eyes to various synophthalmic or cyclopean defects. In figure 2 an embryo is seen with eyes apparently normal, but for their position. They are located on the frontal part of the head in approximation to one another and, while not being fused externally, they are found to be so on examination of sections at a more posterior level. A more intimate approximation of the eyes is to be seen in figure 3. Here the eyes already are con-

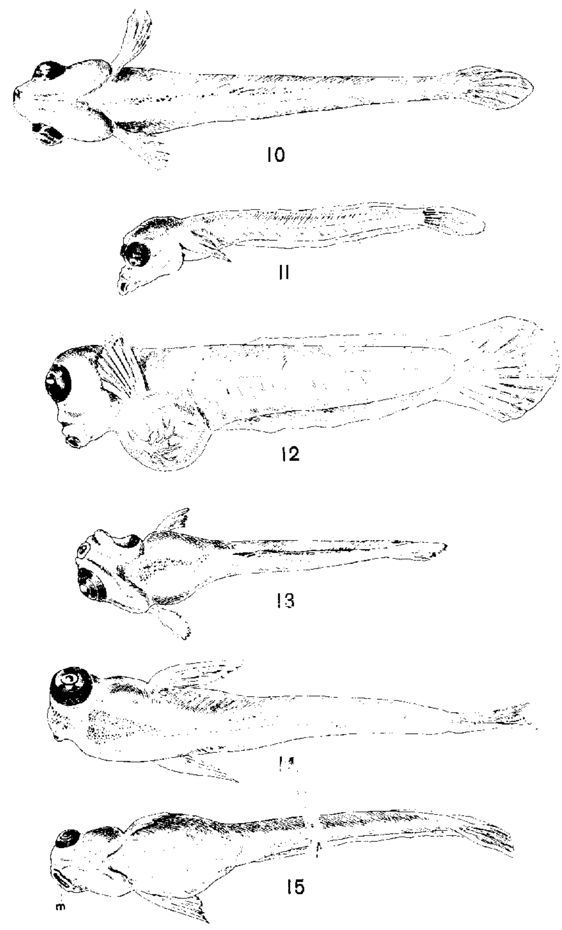


Fig. 10 Normal Fundulus embryo, eighteen days old, five days after hatching.

Fig. 11 Synophthalmia bilentica, from $\frac{1}{12}$ gram molec. butyric acid, thirty-four days old, three days after hatching.

Fig. 12 Cyclopia synophthalmica, from acetone solution (35 cc. gram molec. to 50 cc. sea-water) twenty-nine days old, one day after hatching.

tiguous medially, but not fused. On microscopic examination it was found, however, that there is a fusion of the median parts of the eyes which becomes the more distinct the more posterior the section examined.

In the embryo presented in figure 4 one median composite eye is to be seen, the components facing each other and enclosing one lens.

Cases of cyclopia, i.e. embryos in which the single median eyes on examination in toto do not present any evidence of being composite in character are presented in figures 5 to 9 and 12. A comparison of these figures shows that the cyclopean eye may vary considerably in size as well as in other respects. It may be very large, much larger than a normal eye (fig. 5) or of about the size of the latter or even smaller, and sometimes, indeed, very minute (fig. 8). Furthermore, the cyclopean eye may have the appearance of a normal eye, or it may vary in that respect. Thus, for instance in figure 9 is seen an embryo with a cyclopean eye lacking a lens, but instead showing a very distinct ventral duplicature of the pigment layer, which was verified on microscopic examination. The ectoderm above the cyclopean optic vesicle was probably defective and the optic vesicle has evidently come into too close contact with the yolk, hence the lack of a lens and the duplicature of a part of the wall of the optic cup. Or again, in figure 6 the cyclopean eye of the embryo exhibits a very striking peculiarity of another kind. Here the eye is seen to be irregular in form, anteriorly it lacks the pupil and is entirely surrounded by the pigment layer, while the lens is seen to be on the postero-lateral aspect of the eye.

Besides the synophthalmic or cyclopean defect I have frequently found embryos with a single eye in the usual lateral position of the head (figs. 14 to 17) and of apparently normal

Fig. 13 Embryo with unilaterally defective head, one normal and one rudimentary eye, from acetone solution (35 cc. gram molec. to 50 cc. sea-water; eighteen days old.

Fig. 14 Monophthalmia asymmetrica, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), nineteen days old.

Fig. 15 Monophthalmia asymmetrica, from acetone solution, (35 cc. gram molec. to 50 cc. sea-water) nineteen days old, *m.*, mouth.

structure. As a serial forerunner of this type of monstrosity may be considered embryos in which one eye is normal while the other is rudimentary (fig. 13).

As illustrative examples of variation in the degree of the eye defects may further be presented some cases in which both eyes are of unusually small size (microphthalmic) and often located on the dorsal side of the head (figs. 18 to 21).

b. Defects of the mouth. It is a well known fact that in the cyclopean or synophthalmic embryos of man and other mammals the nose is almost invariably abnormal in shape, structure and

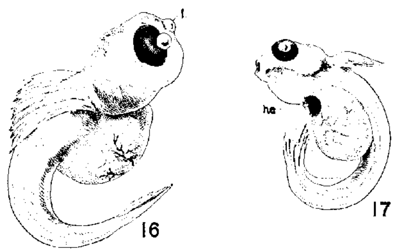


Fig. 16 Embryo with one lateral, malformed eye with free lens, *l.* on eyeless side, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), hatched prematurely on twenty-fourth day after fertilization.

Fig. 17 Embryo with one normal and one heterotopic eye, h. e., from acetone solution (25 cc. gram molec. to 50 cc. sea-water), hatched prematurely on twenty-eighth day after fertilization.

position. It has the form of a proboscis, the nasal passages are more or less blended, a rudimentary septum sometimes being present, while it often may be lacking. Its skeletal parts, if present, consist of cartilage. It is usually situated in the forehead where it hangs down over the cyclopean eye.

In many teratophthalmic embryos of my experiments a very similar deformity is exhibited by the mouth. The malformation is an unusually striking one. The mouth in the normal embryo (figs. 1 and 10) is broad and flattened and antero-median in relation to the eyes which are situated laterally in the head. In synophthalmic and cyclopean (figs. 2, 3, and 12) and sometimes also in asymmetrically monophthalmic embryos the mouth has

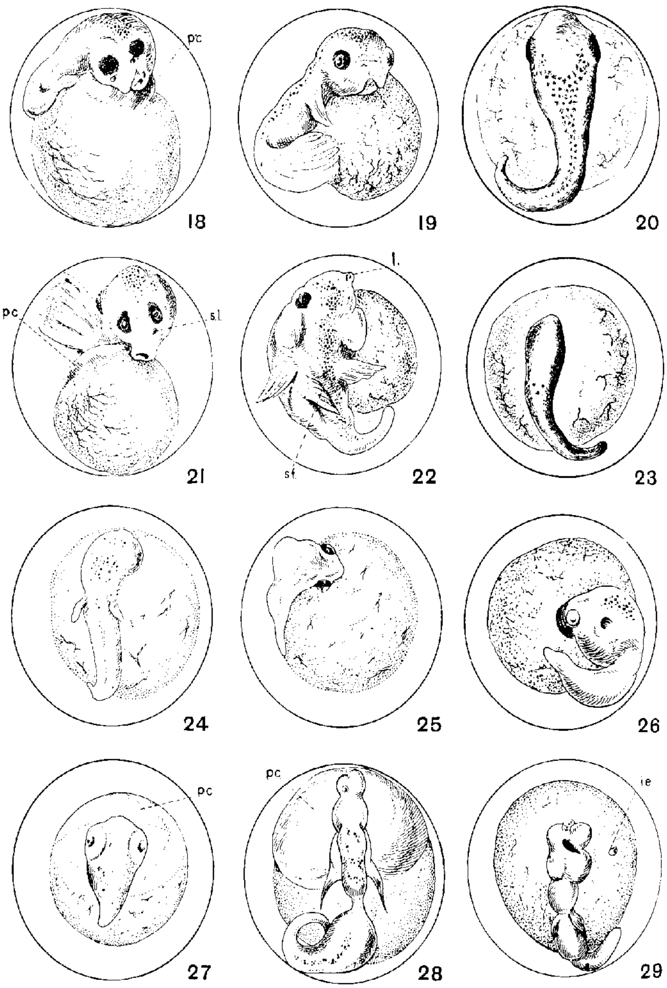
the appearance of an elongated snout or proboscis-like structure. On sections of such embryos it was found that this 'proboscis'-mouth is apparently capable of functioning, for the continuity of the oral cavity with the pharynx and oesophagus is nowhere interrupted.

Stockard ('09) who has also obtained this abnormality of the mouth in cyclopean embryos with magnesium chloride in the same material suggests that

> this condition is due to the fact that the single antero-median eye occupies the position normally assumed by the mouth and thus obstructs the usual forward growth of its structures. The mouth, therefore, remains ventro-posterior to the eye and grows downward, presenting the proboscis-like appearance.

This interpretation of the 'proboscis'-mouth as secondary to the 'cyclopean' condition does not appear to be justified, for I have recorded the occurrence of this anomaly of the mouth not only in cyclopean and synophthalmic, but also in asymmetrically monophthalmic and in some two-eyed microphthalmic embryos (fig. 21). In neither of such cases can the eye or eyes be said to occupy the position which is normally taken by the mouth. Moreover, the very opposite may occasionally be found to occur, viz., that in some asymmetrically monophthalmic embryos (fig. 15) the mouth may take the place which should normally be assumed by the lacking eye. There is good reason to believe that the 'proboscis'-mouth results from approximation and partial fusion of the potential anlagen of the maxillary and mandibular arches, following an injury which has destroyed intermediate parts. This becomes strikingly evident on examination of sections.

Our view is further strengthened by Spemann's ('04) findings who, by constricting amphibian eggs, has produced ophthalmic deformities and the 'proboscis'-shaped mouth in the same embryos. In Spemann's experiments the destruction of material intermediate between the potential eyes, the potential maxillary and mandibular arches could not, by any means, be doubted, for here the relation between a well defined mechanical injury and the resulting morphological defect is evident. Moreover, on exami-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nation of sections of his monsters Spemann ('04, p. 433) finds that the 'Kieferbogenfortsätze' are "*in der Mitte zu einem verschmolzen.*"[4] That the defects of the mouth are syngenetic with those of the eyes is well suggested also by such embryos where the eyes are small and in a very imperfect condition and the mouth exhibits an anomaly analogous to the 'hare lip' or 'cleft palate' of man (figs. 18 and 19).

This syngenesis of defects of the head is further suggested by the

c. Defects of the olfactory pits. The condition of these organs in teratophthalmic embryos is strikingly similar to the defects exhibited by the eyes. All degrees of approximation and blend-

Fig. 18 Microphthalmic embryo with 'hare lip,' from acetone solution (35 cc. gram molecular to 50 cc. sea-water), thirty-one days old, *p.c.* pericardial vesicle.

Fig. 19 Like 18, twenty-four days old.

Fig. 20 Microphthalmic embryo without fins, with club-tail, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), thirteen days old.

Fig. 21 Microphthalmic embryo, with 'proboscis'-shaped mouth, supernumerary lens, *s.l.*, and one pectoral fin only, *p.c.*, pericardial vesicle.

Fig. 22 Embryo with one lateral rudimentary eye, free lens, *l.* on eyeless side, oedamatous ear vesicles and a supernumerary pectoral fin, *s.f.*, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), thirty-four days old.

Fig. 23 Anophthalmic embryo without fins and with club-tail, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 24 Anophthalmic, malformed embryo with rudimentary pectoral fins and club-tail, from acetone solution (20 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 25 Greatly malformed embryo, with small eyes, without fins, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

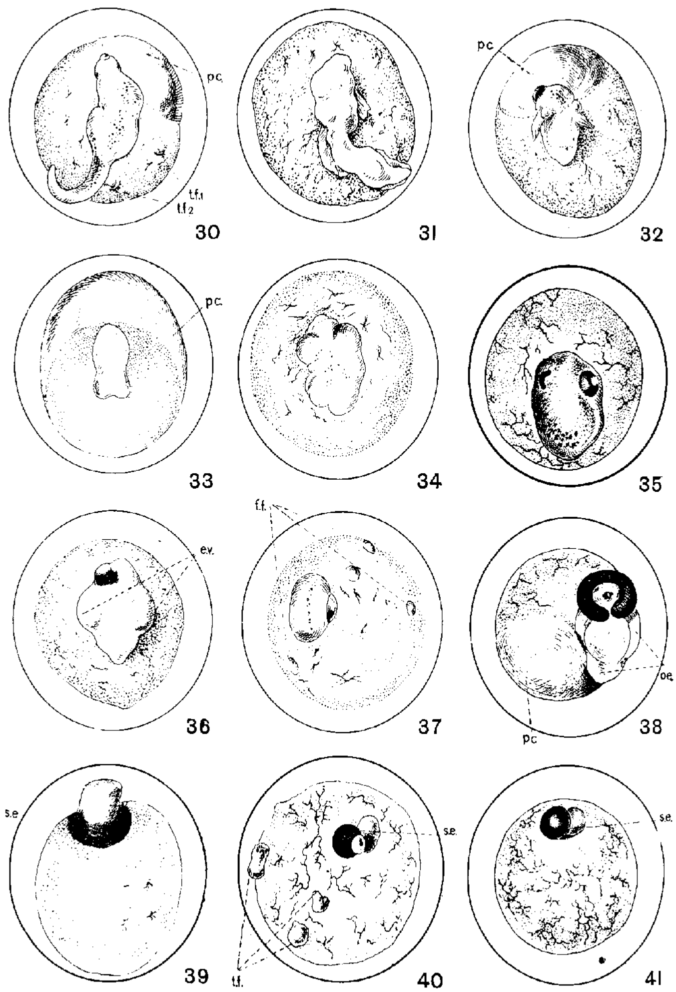
Fig. 26 Amorphous, oedematous embryo, with rudimentary eyes, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 27 Deformed embryo, with short posterior part of body and rudimentary eyes, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old *p.c.*, pericardial vesicle.

Fig. 28 Greatly malformed, elongate embryo with waist-like constrictions, with one vestigial eye and club-tail, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old, *p.c.* pericardial vesicle.

Fig. 29 Greatly malformed embryo, with waist-like constrictions, one median rudimentary eye, rudimentary pectoral fins, club-tail and a very small isolated eye, *i.e.*, on yolk-sac, from acetone solution (40 cc. gram molec. acetone to 50 cc. sea-water), thirteen days old.

[4] My own italics.



pc.
t.f.1
t.f.2
30
31
pc
32
pc.
33
34
35
e.v.
36
f.f.
37
oe.
pc
38
s.e
39
s.e.
t.f.
40
s.e.
41

ing of both pits can be found in various synophthalmic and cyclopean embryos. This observation was made also by Stockard on his 'magnesium-embryos,' but he has not drawn the conclusion to which the data would seem to point. In a subsequent section of this paper dealing with the microscopic anatomy of some terata the attempt is made to account for the teratophthalmic condition, the anomalies of the mouth and olfactory organs on the basis of a morphogenetic factor common to all of these as well as to other deformities in the head region.

d. Defects of the ear vesicles. The deformities to which this organ is subject under the influence of the toxic solutions employed in my experiments, vary considerably. Already on examination in toto of most teratophthalmic or otherwise deformed embryos it can be seen (figs. 2, 6, 8, 22) that the ear vesicles are greatly distended and sometimes reach enormous size. In some embryos there may occur a fusion of both ear vesicles, a condi-

Fig. 30 Amorphous, anophthalmic embryo, with two isolated tissue fragments, *t.f.*$_1$ and *t.f.*$_2$, on yolk-sac; from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old, *p.v.*, pericardial vesicle.

Fig. 31 Amorphous, anophthalmic embryo from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 32 Dwarfed, malformed embryo with one lateral rudimentary eye, from $\frac{1}{12}$ gram molec. butyric acid, eighteen days old.

Fig. 33 Amorphous embryo, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fifteen days old.

Fig. 34 Amorphous embryo from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 35 Meroplastic embryo with rudimentary eyes, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), eighteen days old.

Fig. 36 Meroplastic embryo with one rudimentary lateral eye and oedematous ear-vesicles, *e.v.*, from $\frac{1}{12}$ gram molec. butyric acid, eighteen days old.

Fig. 37 Head-meroplast with rudimentary eye; four small tissue fragments, *t.f.*, on yolk, from acetone solution (40 cc. gram molec. to 50 cc. sea-water) fourteen days old.

Fig. 38 Egg with oedematous eye-teratoma ('solitary eye'), *oe.*, oedema of the brain fragment; from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old.

Fig. 39 Egg with eye-teratoma (solitary synophthalmia), *s.e.*, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), thirteen days old.

Fig. 40 Egg with 'solitary eye' *s.e.*, and three blastolytic tissue fragments *t.f.*, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 41 Egg with eye-teratoma ('solitary eye'), from acetone solution (35 cc. gram molec. to 50 cc. sea-water) twelve days old.

tion known in human teratology as synotia. Not infrequently, however, the ear vesicles may be unusually small, and in such cases on microscopic examination it may be found that the semicircular canals are very defective, rudimentary or diminutive in size or that one or two of them may be lacking altogether.

Not many embryos could be tested for their capacity of maintaining the equilibrium while moving about, since few of them would hatch if the eggs were treated with butyric acid or acetone. However, upon several of them, which did hatch, the observation was made that they could swim only in circular or spiral

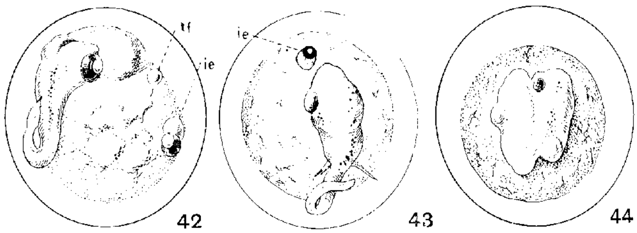


Fig. 42 Asymmetrically monophthalmic embryo, with club-tail, without pectoral fins. On the yolk-sac at a distance from the embryo is seen an isolated tissue fragment *t.f.*, and an isolated eye *i.e.*, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 43 Asymmetrically monophthalmic embryo with isolated eye, *i.e.*, from acetone solution (25 cc. gram molec. to 50 cc. sea-water), sixteen days old.

Fig. 44 Amorphous embryo, in toto making the impression of malformed coalesced twins, from acetone solution (25 cc. gram molec. to 50 cc. sea-water), twelve days old.

lines, or along the wall of the fingerbowl in which they were kept, while they could not move in a straight forward direction, dropping at once to the bottom of the dish, if forced to do so. This functional anomaly agrees well with the structural defects of the semicircular canals spoken of above.

2. The amorphous embryos

This group extends over a wide range of monstrous embryos. It begins with forms which on examination in toto in their shape, size and structural peculiarities only faintly suggest the resem-

blance with fish embryos of a corresponding age, and goes all the way down to such forms where such gross-morphological similarity is entirely obliterated (figs. 24 to 34).

Thus embryos may be found with rudimentary eyes in which the anterior part of the body has widened out to a very striking degree, with club-fins and a club-tail. Not uncommon is the occurrence of greatly elongated and misshapen embryos often with waistlike constrictions (figs. 28 and 29), with rudimentary fins or (sometimes) dislocated fins (figs. 29 and 31). This group would comprise also some remarkably misshapen and dwarfed embryos (figs. 25, 26, 27 and 32) and finally embryos whose form would almost seem to suggest a similarity to some invertebrate animals (figs. 33 and 34).

3. Meroplastic embryos

Very great numbers of eggs were found both in butyric acid and acetone experiments in which parts of the bodies have developed, while the rest of the germ has apparently suffered destruction ('Meroplasts'-Roux, l. c.).

In such meroplastic embryos the same wide range of variation obtains in the degree of defect, as in all other monsters recorded above. Common to nearly all of them, however, is that, usually it is the anterior part of the body that develops, while the posterior part is lacking. The variations in defect concern the organs of the meroplasts as well, as the quantity of what has developed. A glance at figures 35 and 36 shows that the eyes may be variously defective and the same obtains for the ear-vesicles. Likewise the shape and size of the meroplasts are very inconstant. The meroplastic embryo may correspond to more than the anterior half of an embryo or be just about half of the embryo's body. The latter ones recall the hemiembryos which Roux ('95) and later other investigators have obtained by injuring one of the first two blastomeres of the frog's egg.

Finally the meroplasts should be mentioned which are less than half of the body (figs. 37 to 41). Not infrequently all that can be seen to have developed is a more or less malformed head, recognized only by the presence of a rudimentary eye (fig. 37).

Even smaller, usually amorphous, meroplasts can be found in some eggs as the only evidence of development. As the most significant of all the meroplasts recorded may be regarded eggs in which nothing could be found on the yolk-sac besides a very small tissue fragment with an eye ('solitary eye') (figs. 38 to 41). The morphology of such ova and their ontomechanical significance will be discussed under a subsequent heading.

* * *

Of other deformities recorded in these experiments several double monsters would seem to deserve description. Owing, however, to limitations of space, the presentation of these observations is reserved for a paper (soon to be published) which is to deal with a rather large number of various duplicities recorded in more recent experiments.

III. THE MICROSCOPIC ANATOMY OF THE MONSTERS AND CONCLUSIONS REGARDING THEIR MORPHOGENESIS

This part of the work has yielded some very encouraging results. It has, as I hope to make clear, contributed not only a basis for rational interpretation of the morphogenesis of some monsters, but also disclosed some general principles which may underly the genesis of all malformations recorded in my experiments, and possibly of such as occur spontaneously in a state of nature. While for this reason an extensive study of these terata would seem very desirable, I have so far not been able to study more than a few common types. A more extensive treatment of these experimental terata will follow this communication in further studies.

In the present study I have given most attention to the morphology of ophthalmic terata.

A. CLASSIFICATION OF OPHTHALMIC TERATA

There being, as I have mentioned before, a very great variety of these deformities, a classification of them, although of necessity arbitrary to some extent, would seem desirable. For, it is obvious that the term 'cyclopia' in its present use does not

define any one of the conditions which it is meant to cover. A similar objection must be made to the term 'semi-cyclopia' (Gemmill, '12) for, the terata, which this term would define, are of many kinds, and while gross-morphologically similar, they mostly differ in important structural detail. It is also hardly necessary to emphasize that the term 'hour-glass eye,' so commonly employed, is wholly unscientific, for it utterly lacks the precision that is necessary to define a morphological condition.

Fairly serviceable classifications of the various eye monsters have been made by Vrolick ('49), Kundrat ('82) and Bock ('89). However, these classifications being based on mammalian terata and considering not only defects of the eye but also the correlated defects of the nose and skull are lacking in precision (even if applied to mammals only), and do not well lend themselves to other vertebrates. A rational classification which would consider the deformities of both these organs appears to be well-nigh impossible.

I have, therefore, attempted the following simple classification of the teratophthalmic ('cyclopean') monsters with the condition of single- or two-eyedness respectively as the only and basic morphological criterion:

I. Synophthalmia { a. bilentica. b. unilentica.

II. Cyclopia s. Monophthalmia mediana { a. synophthalmica. b. perfecta.

III. Monophthalmia asymmetrica (s. lateralis)

Under 'synophthalmia' will be classified cases of either more or less approximated, closely approximated eyes, or eyes so fused that the composite character of the organ is easily discernible in toto. If such an optic organ should possess two symmetrically placed lenses, the term 'synophthalmia bilentica' will be applied, while, if on more intimate fusion of the eye components only one lens should be present, 'synophthalmia unilentica' will be used as the descriptive term.

The term 'cyclopia' will be applied where a single median eye is present, which on examination in toto does not present the appearance of a composite eye. If, however, such an eye on

microscopic examination of sections is found to consist of two blended eye components, I shall term it 'cyclopia synophthalmica.' To this category belong by far the greatest number of cyclopean monsters.

Of rare occurrence in my experiments (as well as in the cases of spontaneous cyclopia described by various authors) were found to be cyclopean eyes which on microscopic examination were single throughout and nowhere suggesting the possibility of their being composite in character. Such cases I shall henceforth term 'perfect ('true') cyclopia' (Schwalbe's 'cyclopia completa'). In perfect cyclopia the eye is, as a rule, more defective than in synophthalmic cyclopia. It may be very small in size (microphthalmic) or some of its structures may be more or less defective or even lacking entirely. In perfect cyclopia the defects of the brain and other defects are usually much greater than in synophthalmic cyclopia.

The term 'Monophthalmia asymmetrica' has been in use since its introduction by Ahlfeld ('80–'82) and would seem to need no further comments. The embryos of this group possess one eye in the usual lateral position of the head.

B. THE MORPHOLOGY OF TERATOPHTHALMIA

A fairly extensive study of teratophthalmic embryos in sections has enabled me to make many observations which directly or indirectly point to certain dynamic factors underlying their formation. It was found that these embryos sustain at an early stage of development an injury mainly in a restricted area of the anterior end of the future embryo's body which eventually leads to the formation of the terata of the eye. In the following I shall now present anatomical descriptions of various types of teratophthalmia and such evidence will be pointed out as may reasonably be adduced to the interpretation of their morphogenesis. Our description will begin with bilentic synophthalmia, where the defect is yet relatively slight and take up successively the more extreme malformations of the 'cyclocephalic group' through cyclopia all the way down to anophthalmia. The anatomy of asymmetric monophthalmia will also be considered,

which, while being outside of the 'cyclocephalic group,' yet exhibits ample evidence that it owes its formation to like morphogenetic factors.

a. Synophthalmia bilentica. One of the embryos which we have chosen for the presentation of this malformation is seen in toto in figure 3 (p. 488). The eyes are large, not fused, but very closely adjacent. On microscopic examination it is seen that in anterior sections the eyes are separate (fig. 63). If, however, the whole series be examined, it is found that at about the level at which the lens appears in the sections, the medial margins of the eye-bulbs begin to blend; and when followed more posteriorwards this fusion becomes so intimate as to manifest itself in a unition of the retina of one eye with that of the other. The eyes appear to be otherwise normal in structure and two optic nerves are present which are seen to enter the optic lobes after having formed a chiasma (fig. 64).

Examination of the entire series of sections of this embryo suggests that the injury sustained by it which is responsible for its ophthalmic malformation was apparently restricted to the most anterior part of the future embryo's body. This is evidenced by the following data. The abnormalities such as are found to characterize this embryo concern the mouth, the olfactory organ and the most anterior part of the brain, viz., the fore-brain. As seen in figure 3 the mouth is a typical proboscis, and the olfactory pits are in sections seen to be perfectly blended into one large pit (fig. 63). The fore-brain is abnormal in structure and unpaired (fig. 63), while the mid- and hind-brain are bilaterally symmetrical and apparently normal in other respects (fig. 64). On following out the whole series of sections no other abnormality can be detected. The injury sustained is thus very clearly seen to be restricted to the embryo's anterior end. Its probable nature and its morphogenetic consequences will be pointed out in the course of the following description of other embryos where a like teratogenetic principle seems to obtain.

b. Synophthalmia unilentica. The embryo selected for the description of this deformity is from the same experiment as the preceding one and was twenty-four days old when killed. In

toto (fig. 4) it almost made the appearance of a perfectly cyclopean monster, there being only a single median eye present with one centrally located lens. The pigment wall of the eye-bulb, however, was strikingly abnormal, because presenting a figure similar to two C's blended at their opposite ends, it indicated

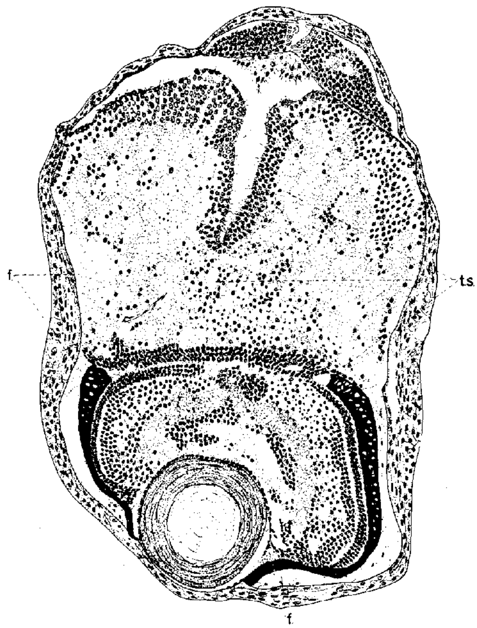


Fig. 45 Camera lucida drawing of a transverse section through the eye of the embryo in figure 4. *t.s.*, tissue spaces; *f.*, fibrin. × 173⅓

the composition of the eye of the ophthalmoblastic materials of both sides.

On microscopic examination of this embryo the following findings were noted. The eye is composed of two incomplete optic cups facing each other and enclosing a single lens of about the usual size (fig. 45). The cornea and iris are apparently normally developed. The anterior chamber of the eye is absent and the

vitreous body only barely suggested. The retina, while being well differentiated, is abnormal in some of its layers, which are to a considerable extent intermingled with the fibrous layer. Some of the retinal layers of the two optic cups are continuous in sections through the anterior part of the eye (fig. 45) while no such continuity can be observed more posteriorly (fig. 46). Two optic nerves are seen to pass out of the eye in few and loose

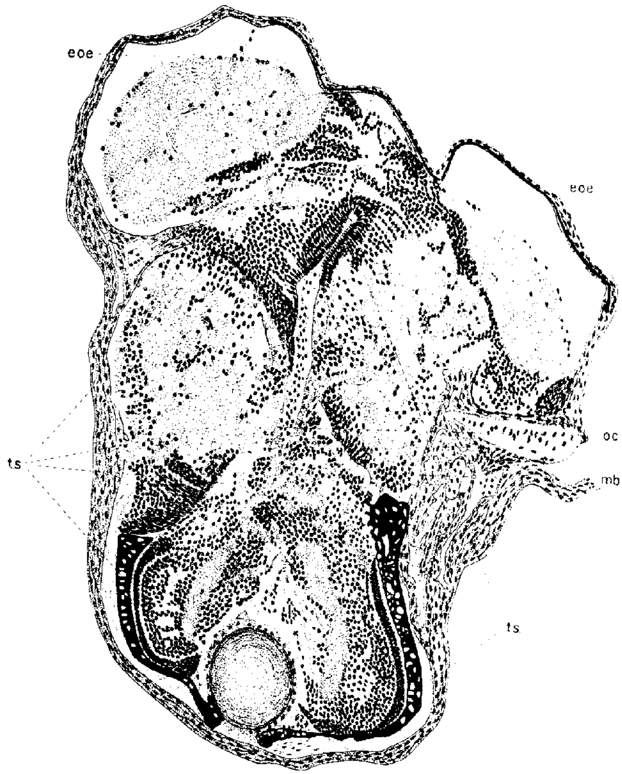


Fig. 46 Camera lucida drawing of a more posterior section through the eye of the same embryo (region of optic lobes). *t.s.*, tissue spaces; *e.oe.*, extracerebral oedema; *o.c.*, optic cross, *mb.*, mandible. × 173⅓.

bundles of fibers and to enter the opposite sides of the brain after having formed an indistinct cross (fig. 46).

The incompleteness of the fused optic cups and the other abnormalities exhibited by this eye are probably due to the circumstance that at a very early stage of development a large part of the ophthalmoblastic material had undergone destructive changes and has suffered partial elimination owing to the chemical action of the toxic solution and physical action (increased osmotic pressure) due to subsequent transfer to pure sea-water. The injury sustained by the embryo has apparently been the severest at the most anterior point of the chief body axis, diminishing gradually posteriorly. The following data would seem to substantiate this interpretation. The malformation is restricted mainly to the eyes and the anterior part of the head. For, examination of sections shows that the fore-brain of this embryo is unpaired and otherwise defective, while the rest of the brain is, when followed in sections posteriorwards, seen to gradually present more and more distinctly the condition of bilateral symmetry. The mid-brain and hind-brain while being bilateral, exhibit, however, a certain other abnormality. The injury here was apparently restricted to the blood and lymph vessels, the earliest anlagen of which seem to have been arrested in their development. This condition can be recognized by the great number of large, clear and empty spaces (figs. 45 and 46) in the tissues of the posterior parts of the brain which in the living embryo have apparently been filled with fluid owing to the existing imperfection in the circulation. A condition of oedema has thus apparently resulted from lack of drainage, an analogue of which is also represented by the oedematous distension of the cranial cavity in the region of the fore-brain and mid-brain. No other abnormalities of the posterior parts of the brain or any other part of the embryo can be noted, which suggests the conclusion that the anterior part of the embryo's body is the most sensitive one and thus subject to the highest degree of injury.

c. Cyclopia synophthalmica. The distinction between this deformity and the preceding one is based on morphological differences existing between the two types of a genetically similar

malformation. These differences are apparently such of degree only, and probably due to differences in the degree of intensity of action of the same morphogenetic factors.

Synophthalmic cyclopia is characterized by the presence in the embryo of a single median eye usually of a larger size than the normal eye, not composite in its appearance in toto but very markedly so on microscopic examination of sections. The following two cases may illustrate this deformity.

In figure 5 (p. 488) is presented an embryo which has a single, very large, well-formed, median eye, which on examination in toto reveals nothing that would suggest its composite character.

On microscopic examination of transverse sections the most anterior sections still present the appearance of a solid non-composite eye, while at about the level at which the lens begins to appear in the sections, the eye-cup discloses its composite character the more the further the sections are followed out posteriorwards, until at the level of the optic lobes this condition of fusion is seen to be very striking. In this region, as well as somewhat anterior and posterior to it, the optic cup presents the appearance of the horizontal section of a funnel (fig. 65). The small end of this funnel is blind and enclosed by the brain, into the substance of which it is seen to dip to a remarkable depth. This funnel-shaped eye is unusually large and the brain is strikingly small. The structures of the optic cup are well differentiated as far as the large part of the 'funnel' is concerned, for here the pigment layer and all layers of the retina are present in their typical appearance. In the small end, however, there are only slight traces of the rods-and-cones layer between the outer margins of the 'funnel' and the brain, while of the other parts of the retina the fibrous and ganglionic layers are present throughout and are seen to be in continuation with these parts of the large end.

The interpretation of the morphogenesis of this cyclopean eye is facilitated by examination of sections of the entire head. The following conditions are revealed by it. The olfactory pits are fused. The fore-brain is unusually short and unpaired, while the mid-brain is bilaterally symmetrical but not distinctly divided

into two hemispheres. This is very striking on comparison of figure 64 with figure 65. Both illustrate sections of approximately the same region. While in the former (the case of bilentic synophthalmia described above) the separation of the two hemispheres is very distinct, in this cyclopean embryo (fig. 65) there is an apparent rupture of the solid brain mass caused evidently by the expansive growth of the eye which it tightly encloses. This is a break rather than a natural division and it can be followed throughout the entire mid-brain, while the hind-brain is distinctly bilobed. No other abnormalities were found in this embryo.

These data suggest that the ovum at an early stage of its development has sustained an injury at the anterior end of the future embryo's chief body axis. The injury apparently consisted in a destructive elimination of a small, very sharply pointed wedge of tissue (the point directed posteriorwards), comprising the future interocular area, possibly a small part of the opthalmoblastic material, and, evidently, also a considerable part of the future brain. The coalescence of the wound surfaces has caused an approximation and subsequent fusion of parts, which in turn eventually resulted in cyclopia.

The other case of synophthalmic cyclopia concerns an embryo (fig. 12) (p. 490) whose head exhibits some striking features. It is relatively very small (microcephalus), the mouth is a wide open, typical proboscis and the eye is single, median in position, very large and betrays on examination in toto no evidence whatever of being composite in character.

In anterior sections of this embryo (fig. 66) the appearance of a normal, transversely sectioned, eye is presented, and the synophthalmic character is revealed only by examination of sections at a more posterior level. However, if the brain is examined in anterior sections the nature of the process is disclosed to which the malformation secondarily owes its origin. The forebrain is unpaired and very small, for, in size it hardly exceeds that of the eye. The most remarkable feature, however, which the brain at this level presents, is a fragment of retina which is fused with it at its lower extreme right and just above the orbit

of the eye. The significance of this retinal fragment is at once recognized when the condition of the whole eye is considered. On following the sections posteriorwards the eye more and more appears to be oval in shape. More posteriorly yet, the eye widens out enormously (fig. 67) while the brain is at this point very distorted and strikingly small in size. A few sections further posteriorly, the shape of the eye is still practically the same, but its size has diminished somewhat while that of the brain has increased. The latter which is now in the region of the optic lobes is very distinctly bilobed, the right hemisphere, although somewhat distorted, is, however, complete, while of the left one about a half is wanting, the place of this lacking part of

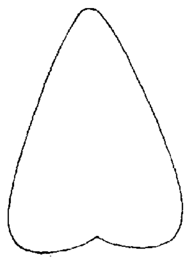


Fig. 47 Diagrammatic outline reconstruction of the cyclopean eye of the embryo in figure 12.

the brain being occupied by the larger part of the eye, which at this level is horse-shoe-shaped in cross-section. The very last sections of the eye prove unmistakably that it is composite, for its base consists of two separate optic cups.

The nature of this eye is best understood from the diagrammatic outline reconstruction which is attempted in figure 47. It is a heart-shaped body with the apex directed frontalwards and the base cerebralwards.

The question now arises in what way this peculiar malformation came about. The intracerebral retinal fragment which was referred to above (fig. 66) as well as the shape of the eye and the defects of the brain point to an answer which, I think, contains a high degree of probability. Here, too, the injury sustained by

the embryo was restricted to its most anterior portion. Owing to this lesion resulting from a process of destruction which I term blastolysis, a wedge of blastema (with the sharp point directed posteriorwards) was eliminated, and the subsequent coalescence of the wound surfaces has caused the earliest optic anlagen to fuse. The eliminated wedge-shaped piece of tissue contained apparently the future interocular area, much more of the anterior parts of the ophthalmoblastic material of both sides than of their posterior parts, and a part of the potential brain. This would account for the perfect fusion of the anterior part of the eye components, as well as for the incomplete fusion of their posterior parts, of which probably very little had been lost. That the process which caused the injury was apparently one of dissociation and dispersion (blastolysis) would seem to be evidenced by the retinal fragment, which can only secondarily have come to fuse with the brain, i.e., after the ophthalmoblastic fragment which has given rise to it had been dislocated cerebralwards from its natural position.

d. Cyclopia perfecta. This monstrostity represents a very high degree of ophthalmic malformation. In its morphogenesis it differs from synophthalmic cyclopia, for the perfectly cyclopean eye is genetically a single eye. However, the evidence which I have been able to find, points to the same dynamic process, namely, blastolytic action of the altered environment as the factor responsible for its formation.

A few examples may now be presented.

In figure 6 (p. 488) is presented an embryo which is possessed of a single median eye and small defective fins, partly obscured from view by the very large oedematous ear vesicles. The whole body is distended and the tail is finless and club-shaped.

The examination of the eye discloses a very remarkable condition. Already in toto (fig. 6) the eye is seen to be abnormal in shape and in the position of the lens, which is situated posterolaterally, instead of being anteriorly and in the center of the eye. The front of the eye is entirely closed over by the pigment layer, so that the condition presented might almost be considered as a rotation of the polar axis of the organ.

The microscopic examination of the sectioned embryo confirms the macroscopic appearance. The whole eye appears in forty-nine sections of 6μ thickness. Up to the twenty-second section no trace of a lens is seen, and in these anterior sections the condition is presented which normally obtains for posterior sections, i.e. the view is one of the base of an eye where it is entirely encircled by the pigment layer. In the section illustrated by figure 68 of Plate I the retinal layers are defective and the brain, while being bilobed, yet appears to be greatly distorted. Following the sections posteriorwards we see in the twenty-second section (fig. 69) the beginning of the lens in its abnormal position. It extends up to the forty-seventh section inclusively and it is thus seen to occupy about the posterior nine-sixteenth of the eye. No optic nerve can be found, although the fibrous layer of the retina is fairly well developed. The mouth is absent in this embryo and the pharynx comes into view in the last sections through the eye, which is seen to be partly enclosed by the defective mandibular arches and projecting for the greater part into the distended pericardial vesicle.

The optic cup is C-shaped; it strikingly suggests the similarity to a component of a synophthalmic eye and points to the morphogenesis of this monstrosity. Owing to blastolysis the future interocular area of the early embryo, the entire ophthalmoblastic material of one side and a part of it of the other side were destroyed as well as also the earliest anlage of one olfactory pit. The subsequent approximation of the wound surfaces has moved the remainder of the one uninjured ophthalmic anlage out of its original position so that the incomplete optic cup which has developed from it, has turned at an angle of about 90° in relation to its axis. The apparent heterotopia of the lens and the pigment-enclosed front of the eye are evidently due solely to this secondary change in position of the remaining part of one optic anlage. No traces of a fusion could be found anywhere, and the presence of a single (non-fused) olfactory pit (fig. 68) would seem to strengthen the evidence that the cyclopean eye of this embryo has developed from the ophthalmoblastic material of one side only.

The injury sustained by the embryo was a severe one, much severer than is usually found in either synophthalmia or synophthalmic cyclopia. This is evidenced by the defective and oedematous condition of the brain (fig. 69), and the curving of the head, to which is due the appearance in one section of the eye, medulla, and semicircular canals. Yet the morphogenetic factor which brought about the embryo's deformity was, no doubt, the same as in synophthalmic monsters.

A case of perfect cyclopia, where the malformation is far more extreme than in the preceding one is presented in figure 8 (p. 488). The embryo is seen to be extremely deformed. It has a single, median, unusually small eye, and greatly distended ear vesicles (cf. fig. 71); the entire body is oedematous, all fins are lacking and the tail is club-shaped.

On microscopic examination (fig. 70) the eye is seen to be genetically single, there being no indication whatever of its having been formed out of optic anlagen of both sides. It is very rudimentary in structure, the pigment layer and the relatively very large lens being its best developed parts, while the retina is very defective, the optic nerve, the iris, anterior chamber and the vitreous body lacking altogether. The brain is lateral to the optic cup of this minute eye instead of being dorsal as should be expected from the position of a cyclopean eye. This distortion of the relation between the eye and the brain, the defective and unpaired condition of the latter throughout (cf. fig. 70) and the deformities of the rest of the body suggest that a severe injury was sustained by the entire embryo. However, the defects are most extreme at the embryo's anterior end, which again points to the conclusion that in this case, too, the degree of injury was the highest at the most anterior part of the early embryonic anlage, diminishing posteriorly along the chief body axis. The eye arose from a fragment of one potential optic anlage, the remainder of which and the entire other potential optic anlage as well as a part of the future brain and the potential olfactory pits having suffered destruction. Owing to subsequent processes of regulation the surviving ophthalmoblastic fragment has come to occupy the median position in the defective head where it developed into the rudimentary eye.

One more case of perfect cyclopia may now be described. On examination of the embryo in toto (fig. 7, p. 488) it was seen that the cyclopean eye is lacking the lens. This may be attributed to the circumstance that the optic vesicle came into contact with damaged head ectoderm.

On examination of sections (fig. 48) conditions are found which again point to blastolysis (dissociation and dispersion) and subsequent regulation as the factors responsible for the formation of perfect cyclopia. The retina is defective, its rods-and-cones layer being fairly well developed on one side and less so on the other side of the optic cup. The ganglionic granular and fibrous layers appear to be scattered and intermingled. Several insular accumulations of retinal cells surrounding fibers can be observed resembling the 'retinal rosettes' recently described by Nehl ('14). The oedema evidenced by numerous large tissue spaces in the eye and in the brain (which latter is highly defective and unpaired), and the distension of the cranial cavity are very likely due secondarily to blastolytic action, the blood vessels, owing to destruction of embryonic material, having failed to develop into a continuous system of drainage. Nothing can be seen in any of the sections that would indicate a fusion of two optic anlagen. Only one optic nerve is seen to pass out of the eye and enter the brain. The complete absence of the olfactory pits and the mouth (the latter coming into view in sections behind the eye) also strengthens the evidence for blastolytic action (dissociation and dispersion) of the environmental modification employed in the experiment. Owing to this action, evidently, the ophthalmoblastic material of one side has been destroyed, while that of the other side has, through subsequent reparation, come to occupy a median position.

e. *Monophthalmia asymmetrica.* I shall now attempt to show that the same morphogenetic factor (blastolysis) is responsible also for the genesis of other cases of teratophthalmia. They are exemplified by embryos in which both eyes are present in the typical later position in the head, one of them being normal, while the other is small in size and rudimentary in structure, by embryos in which one of the eyes is dislocated (ophthalmic ectopia), and by asymmetrically monophthalmic embryos.

The first case now to be described is that of an embryo with one small rudimentary eye, the other eye being apparently normal (fig. 13, p. 490). The embryo is very small as compared with normal embryos of the same age after hatching, its head is curved towards the side of the rudimentary eye, but no other abnormalities could be noticed.

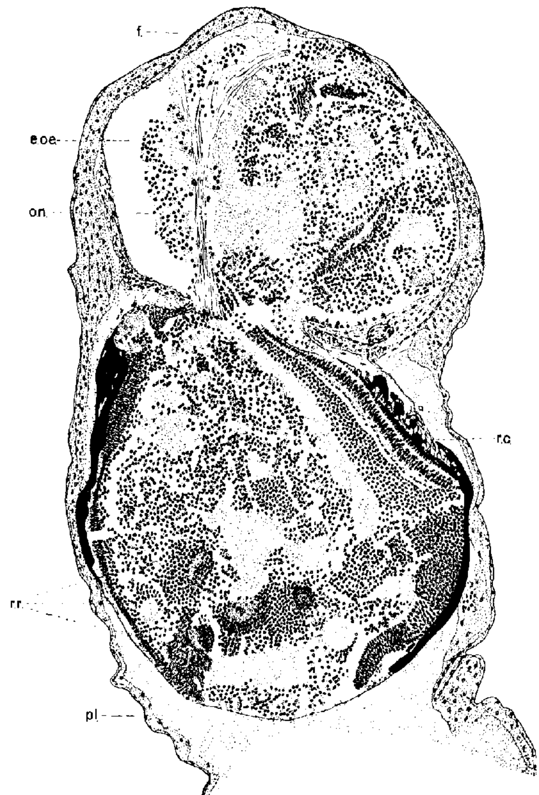


Fig. 48 Camera lucida drawing of a transverse section through the eye region of the perfectly cyclopean embryo in figure 7. *r.c.*, rods and cones; *r.r.*, 'retinal rosettes'; *o.n.*, optic nerve; *f.*, fibrin; *e.oe.*, extracerebral oedema; *pl.*, plasma in pericardium into which the eye dips. × 173⅓

In microscopic sections the embryo's right eye appears to be perfectly normal in every respect, while the left eye appears in the sections posterior to it and extending into the yolk-sac, is diminutive in size, but well differentiated in essential structural details. The optic nerve of the small eye is seen entering the brain independently of the same nerve of the other (normal) eye.

On examination of the entire series of sections the probable nature of the injury sustained by the embryo is gradually disclosed. It is restricted mainly to one side and the abnormalities concern the olfactory pit, the eye, the brain, and the ear vesicle.

While the olfactory pit of the uninjured side is normal and in its typical position antero-median to the normal eye, there are three olfactory pits on the abnormal side, all of which are closely approximated (fig. 72). This points to a fragmentation of the potential rhino-ectoderm at a pre-differential stage of development. The condition of the eye of this side has been described; its probable manner of formation is suggested by a careful examination of the brain. The latter in successive sections is found to consist of two hemispheres, but is strikingly asymmetrical in regard to the position occupied by them in relation to the chief body axis, the right hemisphere preceding in all sections the left one which is pushed posteriorwards (fig. 73). This agrees well with the posteriorward dislocation of the eye on the same side and is probably due to regulation after a sustained unilateral lesion. Striking evidence of a process of dissociation (blastolysis) is found on examination of sections at the level of the optic lobe of this side (fig. 74). The latter, which, like the whole hemisphere of this side, is posterior to the one of the right side, is seen to be at the same level with the posterior part of the left (smaller) eye, with the right ear, the heart, and the yolk of the body cavity. It is incomplete, and looks as if a part of it had been broken off. To the right from it (in the figure) there is a wide cleft. Between it and the head integument there is a large fragment of tissue (o. l. f.) which has the appearance of a small optic cup at an early stage of differentiation, but on careful examination of all sections is found at its most ventral point to be in connection with the optic lobe. This tissue fragment must

thus be considered as a part of the optic lobe, from which it was for the greater part delaminated. The ear of the left side is lacking entirely, and it stands to reason that its absence is due to blastolytic elimination of the otoblastic material of this side.

Very striking evidence of blastolysis can be observed in the embryo in figure 17 (p. 492). It hatched prematurely on the twenty-eighth day, when it was drawn and killed. The yolk-sac is still very large and the embryo is curved. Only the left eye is in the usual position in the head while the right eye is found to be strangely dislocated. It is seen extending between the right mandibular arch and the yolk, in which it is well imbedded, covered by the yolk-sac.

Examination of sections reveals the following conditions. The left eye is well differentiated, but not quite normal. It lacks the anterior chamber and the vitreous body, while the iris is rudimentary. An optic nerve is present and can be followed out to enter the hemisphere of the opposite side. The brain is apparently normal everywhere. In the sections at the level of the medulla and semicircular canals there comes into view the heterotopic eye (fig. 75). It is enclosed between the yolk and the yolk sac and is in proximity to the heart. Between the heart and the dislocated eye very large lymphocytes are seen. The eye is as well differentiated as the orthotopic left eye and is oval in shape, which may perhaps be due to its extra-orbital development. It lacks a pupil and the vitreous body, while the iris also appears to be defective. No optic nerve could be found.

An important clue to the genesis of these malformations is furnished by the condition of the olfactory pits. The latter can be followed out very satisfactorily in the most anterior sections. Antero-median from the left (orthotopic) eye there is one olfactoroy pit—in its normal position. On the right side, however, (where the eye has been dislocated) there can be seen in sections somewhat more posterior, as many as four, minute olfactory pits. It seems obvious that these four small olfactory pits have developed from four fragments of the dissociated potential anlage of the olfactory pit of this side. Dissociation

has, then, on this side affected both the potential olfactory and eye anlagen, fragmenting the one and delaminating and dislocating the other. The effect is an increased number of olfactory pits and ophthalmic heterotopia.

The same morphogenetic factor was apparently responsible also for the conditions found in the embryo in figure 16, p. 492. Its head is unusually large and suggests oedema. The left eye is lacking entirely and a free lens is found in its place. The right eye is defective, the optic cup being C-shaped and the large lens greatly protruding, owing to the absence of an anterior chamber.

In the most anterior sections only the 'independent' lens is seen which is not yet fully differentiated. In the next sections there comes into view the lens of the right eye and two olfactory pits. The latter are observed to be so closely approximated as to be partly contingent on their median borders, and to form an angle of about 90 degrees. More posterior sections show that the optic cup of the eye is only anteriorly C-shaped, while it is complete, though small, in its posterior part. The part of the section where the (lacking) left eye should be, is occupied by very loose mesenchyme the interstices of which are filled with plasma. The retina is very well differentiated and one optic nerve can be traced to its entrance into the brain hemisphere of the opposite side. A very remarkable feature is presented by the sections through the base of the eye (fig. 76). Here, ventral to the brain and lateral from the eye from which it is entirely separated, there can be observed a very small optic cup-fragment with all layers (pigment and retinal-rods and cones) perfectly differentiated. This is evidently a dislocated but fully differentiated remnant of the destroyed ophthalmoblastic material of the left side.

Essentially similar conditions were found in the embryo illustrated by figure 15 (p. 490). A ventral view of it is presented in which it can be seen that the right eye is lacking and its place is occupied by the mouth. The left fin is about half the normal size, and no other abnormalities were found on examination of the embryo in toto.

The microscopic examination of sections revealed on the left side (right side in the illustration, fig. 77) a practically normal eye. On the right side in the place of the eye is seen the mouth cavity. The ophthalmoblastic material which was to form the right eye has been largely destroyed and only a small fragment of it has been left, which has developed into an optic cup-fragment, seen enclosed in the cranial cavity at the base of the brain. This fragment of the optic cup shows all layers, including the fibrous layer, of the retina well differentiated.

The brain is bilobed and no abnormalities can be found in it excepting its oblique position in the head with regard to the main body axis. This is either secondary to or syngenetic with the herterotopia of the mouth, which in turn is due to the destruction of the ophthalmoblastic material of one side. The elimination of the latter has allowed the mouth to expand in the direction of least resistance so far, as to occupy the exact position of the lacking eye, while the excessive expansion of the mouth on this side may have caused distortion of the brain in its relation to the body axis.

Very interesting conditions are found also in the embryo, a dorsal view of which is presented in figure 14 (p. 490).

The left eye is lacking and there is a very distinct invagination where the eye should be. On examination of the ventral side the mouth could be seen to be of a shape approaching the 'proboscis' type. Its position was very near to what was to be the place of the lacking eye. No other abnormalities could be observed in the embryo in toto.

Microscopic examination of sections reveals a normal olfactory pit and normal eye on the right side. In sections through the posterior third of the eye (fig. 78) the mouth appears almost exactly in what was to be the place of the eye. An unusually small left olfactory pit comes into view at this level and a minute 'independent' lens is noted on the maxilla. Two more minute lenses are found on sections still more posteriorly (fig. 79). The origin of these lenses on the eyeless side I am inclined to consider as due to contact of remnants of the destroyed ophthalmoblastic material of this side with the ectodermal epithelium.

The brain is bilobed, but asymetric in regard to its relation to the chief body axis, the hemisphere of the side possessing the eye preceding in sections that of the side lacking the eye.

The lesion sustained by the early embryo was evidently restricted to the left side of its anteriormost part, where it has eliminated the entire ophthalmoblastic material, minute remnants of which have apparently stimulated the differentiation of free lenses. Owing to subsequent processes of regulation, the injured side of the head suffered a posteriorward displacement. This would account for the small size and unusual position of the left olfactory pit as well as for the asymmetry in the position of the left hemisphere of the brain.

f. Microphthalmia and Anophthalmia. Microscopic examination of sections of embryos with very small, rudimentary eyes, or such in which no eyes can be detected in toto have likewise disclosed conditions which point to the action of the same dynamic factor that was found to underly the formation of all other eye terata described above.

The following two examples may suffice:

On cross sections (fig. 80) of the microphthalmic embryo illustrated in figure 20 (p. 494) the following view is presented. One eye, while being very small, is seen to be fairly well developed, the retinal layers of the optic cup and the lens being well differentiated. The optic nerve of this eye is very clearly seen to enter the optic lobe of the opposite side. The other eye is much more defective. It consists of a rather poorly differentiated optic cup enclosing a lens. One side of the wall of this optic cup is invaginated and partly surrounds another lens. Medianwards from and near to this eye is an undifferentiated mass of apparently ophthalmic tissue with a large well differentiated lens.

The embryo then, as we see, possesses virtually three eyes, one of which in structure approaches the norm. Of the other two eyes the first is poorly differentiated and possessed of two lenses, while the second is represented by an undifferentiated optic vesicle with a lens.

The ophthalmoblastic material of both sides has suffered lesions, which resulted in the breaking up of one optic anlage

into two and in the small size of the other eye due to destruction of a part of the potential eye anlage of this side. The injury sustained was a rather severe one for it affected also the brain, the bilaterality of which is obscured (as seen in fig. 80), and the rest of the body. However, it is at the anterior end of the embryo's body where most damage seems to have resulted from the process of destructive dissociation (blastolysis).

The general defects are usually even more extreme in embryos in which on examination in toto only a small rudiment of an eye, like a fragment of the pigment epithelium, is found, or where no eyes at all can be detected (cf. figs. 23 and 24). As a rule,

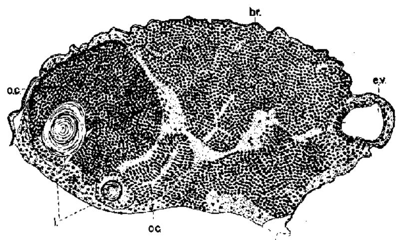


Fig. 49 Camera lucida drawing of a transverse section through the head of the embryo in figure 23. *o.c.*, optic cup; *l.*, lens; *br.*, brain; *e.v.*, ear vesicle. × 125.

it is found on microscopic examination of sections that most anophthalmic embryos possess poorly differentiated and deeply buried eye anlagen, sometimes with a profusion of very small lenses. In figure 49, which is a transverse section through the anterior head region of the embryo in figure 23, two optic vesicles of unequal size can be seen with lenses of corresponding sizes. The simultaneous appearance in the same section of a rudimentary ear vesicle, the unequal size and proximity to each other of the rudimentary optic cups as well as the distortion of the brain would seem to well warrant the assumption of blastolysis as the morphogenetic factor responsible for the defects of this embryo.

C. THE MORPHOGENETIC FACTORS UNDERLYING THE ORIGIN OF EYE TERATA

1. Stockard's inhibition theory

The analysis of the morphogenesis of ophthalmic terata has been attempted repeatedly and with varying success. Good reviews of the opinions of the earlier writers on the subject were already given by Spemann ('04), v. Hippel ('09) and Schwalbe and Josephy ('13), and to these the reader may be referred. No distinction is made by most authors between the various degrees of the synophthalmic condition and cyclopia, i.e. the presence of a single median eye. Thus, their interpretation of the morphogenesis of cyclopia, applies to the whole so-called 'cyclencephalic' group.

Two views have been advanced to account for the morphogenesis of these monstrosities. According to the one represented by Huschke ('32), Dareste ('91) and very recently advocated by Stockard ('09, '10 a, '13) 'cyclopia' is a condition in which the separation of what they consider the originally single optic anlage has been inhibited.

Opposed to this view is the theory of fusion of two optic vesicles as underlying the formation of 'cyclopia,' which was originally advanced by Meckel ('26), and which in a modified form has recently been advocated by most investigators of the subject and particularly by Spemann.

This author (Spemann '03, '04) has with an entirely different object in view constricted eggs of Triton taeniatus by placing in the two-cell stage a ligature around the first cleavage furrow in relation to which it was somewhat oblique. As a result of this operation he obtained embryos in which the anterior end was doubled to a greater or less degree, depending upon the degree of constriction. In many of the embryos thus treated Spemann observed that one of the doubled heads (or parts of the head) thus resulting was normal while the other which, owing to the oblique ligature was narrower, had defective, usually synophthalmic and sometimes cyclopean eyes. Since the eye deformity was mostly found on the part distal from the embryo's

main body axis, Spemann ('03, '04) concluded that the 'cyclopean' deformity produced by him was due to a defect because it resulted from the destruction of the area which would normally be the area between the eyes. While not inclined to support unreservedly the hypothesis originally advanced by Meckel ('26), namely that the synophthalmic or cyclopean condition results from a secondary fusion of two originally separate optic vesicles, Spemann leans very strongly ('04, pp. 440–441) to the view advanced by Fischel ('03) according to which cyclopia might result from a fusion *at a very early stage of development of two originally separate masses of cells which were to form the eyes but had fused before they began to undergo the process of differentiation* into these organs. With this latter view agree well the observations made by Stockard ('09) which I can confirm from my own experience and which were confirmed also by Lewis ('09), namely that the synophthalmic and monophthalmic deformities can be recognized as such already in the stage of the optic vesicle, in other words that a 'twin optic vesicle' or 'cyclopean optic vesicle,' if these expressions be permitted, comes off from the brain directly as such.

The experiments of W. H. Lewis ('09) have also a very important direct bearing on the subject of morphogenesis of teratophthalmia. This author employed the method of pricking the anterior end of Fundulus eggs in the embryonic shield stage. Various synophthalmic and one-eyed monsters resulted from these operations, depending on the degree and exact localization of the injury inflicted.

From these results Lewis concluded that in the cases of 'cyclopia' certain cells have been destroyed by pricking, which would normally form the area between the eys. Owing to this elimination of tissue, "the repair, taking place after the operation, consists of a closing together of the parts left behind . . . . and rudiments are thus brought into contact that normally are quite widely separated, those of the eyes, for example." This is essentially an assumption of a fusion of early optic anlagen before their differentiation into optic vesicles, as underlying the morphogenesis of cyclopean and synophthalmic monsters.

Very recently Mall ('08) and Whitehead ('09) have advanced essentially identical views in confirmation of Lewis' ('09) conclusions.

Thus, as we see, the recent authors practically all agree upon the mode of formation of the 'cyclopean' eye. However, the fusion theory of 'cyclopia' does not altogether lack opponents, as the most ardent of whom we must now regard Stockard.

For a critique of this author's views the reader must be referred to Spemann's ('12 a and b) excellent discussion. While on the basis of evidence in my possession much could be added to the latter, I shall for the present confine myself largely to the discussion of the arguments which Stockard has most recently ('13) brought forth in defense of his views on the morphogenesis of 'cyclopia.'

Concluding from his experiments with magnesium chloride on Fundulus eggs and also from experiments with alcohol, ether, and chloroform-acetone solutions on the same material and with very similar results, Stockard ('09, '10a, p. 387) concluded that "the evidence strongly indicates that the ophthalmic abnormalities produced in these experiments are the result of an anaesthetic action during the early developmental stages."

The fallacy of this hypothesis of anaesthetic inhibition has become obvious since the work of McClendon who has recently ('12 a) shown that 'cyclopia' and other malformations of the eye can be produced by various other, non-anaesthetic substances. Thus in his most recent publication, Stockard ('13) no longer speaks of '*anaesthetic*[5] action,' but instead he considers his experimental eye terata as "the result of a *weakened*[5] development" which is brought about by the toxic solutions. These solutions, he argues, "all tend to suppress or arrest the development of the eye material in the brain." ('13, p. 271).

Stockard ('13, p. 254) believes

> that the eye anlage in the medullary plate is primarily median and single, and normally separates into two almost equal growth *regions*, which develop in lateral directions reaching further and further out

[5] My own italics.

until finally the optic vesicles come into contact with the ectoderm at the sides of the head.

On the basis of this hypothesis he now ('13, p. 273) offers the following explanation for the morphogenesis of synophthalmic and one-eyed monsters:

> . . . . the median eye anlage does not widen or spread laterally but is arrested in its primary condition; thus the growth centers are not sufficiently separated and only a single center exists, and even more than this, the arrest is to such an extent that the entire or normal amount of optic material does not differentiate. Hence one finds a median cyclopean eye consisting of an amount of eye material far below that normally present.

This for cyclopia. In the various degrees of synophthalmia he assumes that the 'developmental vigor' is less suppressed, less 'weakened.' Here the separation of the single anlage into two 'growth centers' is inhibited only to a certain (varying) degree and depending on the variation in the degree of inhibition various synophthalmic conditions, such as the "cyclopean eye showing distinctly its double composition," the "hour-glass eye or incomplete cyclopia," approximation of two separate eyes, etc., result.

For the genesis of lateral monophthalmia, finally, Stockard ('13) makes the following suggestion:

> The growth centers representing the two future eyes of an individual are rarely equally vigorous. . . . . It might be that at some critical point in development one of the future eye centers is affected after the growth centers had begun to localize in more or less lateral positions.

It is very difficult to understand why (in the same experiment!) in some embryos the inhibition of one of the potential eyes should begin after the division of the single anlage (monophthalmia asymmetrica), while in other embryos this single eye anlage should be inhibited before its division into two parts takes place ('cyclopia').

That the latter is primarily single and median in position in the medullary plate Stockard now regards as a fact, which he thinks he has established by experiments described in his 1913 paper. However, it seems more than probable that the method

which he employed in removing fragments of tissue from the antero-median and the antero-lateral portion of the medullary plate has led to errors which Stockard apparently must have overlooked. The unavoidable inaccuracy inherent in the method of mechanically removing minute fragments is obvious. Even with such refined methods as Spemann has employed and with the experience and skill of the latter in such operations, this inaccuracy can not be entirely avoided, as Spemann himself has repeatedly pointed out. It must also be borne in mind that the whole area from which the eyes can presumably arise, is in the stage, at which Stockard performed his removal operations, relatively very small. How could any satisfactory degree of precision be attained in operations performed on this small area with 'fine scissors' (Stockard's method)? Is it not probable, that while cutting out the antero-median region of the medullary plate, Stockard has evidently removed also lateral material? Or, that, while attempting to cut away antero-lateral parts of the medullary plate he evidently removed too little, being anxious to avoid inaccuracies resulting from cuts carried too far from a presumably correct position? In the medullary plate, the region under discussion is—at least on superficial examination—morphologically homogeneous, and the mapping out of morphogenetic areas for analytic experiments is beset with well-nigh insurmountable difficulties, even if the instrument used for the operation be the finest conceivable. This can readily be seen from the results which Stockard reports to have obtained from his experiments. Of nine embryos in which 'narrow strips' were removed from the antero-median part of the medullary plate, "four . . . . failed entirely to develop eyes." Of the five other embryos only in one the eyes developed "to an extent approaching the normal" and "four . . . . individuals possessed highly defective eyes" ('13, p. 288).

Do such results warrant any conclusions at all? Are not the four cases, where defective eyes resulted from the removal of the antero-median tissue, at least as conclusive for the lateral position of two optic anlagen in the medullary plate as the four eyeless embryos for the median position of a single optic anlage?

Is it not safe to hold that in these experiments the results can not be read aright, owing to the great probability of an error introduced by the inadequate method of the experiment? It would seem obvious that, if experiments be performed on the said area at this stage of development with an instrument as crude as the one employed by Stockard, the deductions from the results are bound to be either erroneous or at least very unsafe in almost every case.

For the validity of this claim of the 'single eye anlage' and its 'median position in the medullary plate' Stockard ('13, pp. 274–276) attempts another proof. He holds that the position of the optic cross outside and below the brain would be inconceivable, if the optic anlagen should be lateral in position and other brain tissue be present between them. For, then, he concludes, the optic stalks instead of having a "median origin and connection" would be "attached to lateral regions of the brain from which the optic vesicles pushed out." According to his diagram presented in figure 8 (p. 276) ". . . . in the course of development the fibers of the optic nerve following the stalk reach the lateral position and must enter the brain and continue within its tissue in order to meet the nerve of the opposite side and form the cross or chiasma. Brain tissue would lie beneath the optic chiasma" and "this condition is never found in any normal vertebrate."

These deductions would have to be regarded as very important if they were correct. But, as will be seen from the following, they will not hold good.

At the outset it should be said that the optic vesicles do not 'push out.' Instead they are being pushed out by the primoridum of the brain. The parts of the brain anlage (and not of the eye vesicles, as Stockard suggests) most directly concerned in this process of pushing out the anlage for the eye, elongate more and more, until they have attained the form of optic stalks at the time when the differentiating eye vesicles have reached their final positions in the head. Their origin is just as much from tissue dorso-lateral as from ventro-median to the optic vesicle. It is true that this view is, as yet, not based on experi-

mental evidence, but even lacking this important basis, it would seem far more reasonable and safer and far less arbitrary than the unwarranted claim that the 'optic anlage' is median and that the optic stalks are a part of it. Granting, however, that my view is correct—and there is at least a very high degree of probability in it—it is easy to understand that optic stalk tissue may be partly median in origin while the optic vesicles come from antero-lateral regions of the medullary plate. In this case, then, it is also evident that the position of the optic stalks and later of the optic nerves and chiasma ventral to and outside of the brain can not, by any means, be regarded as evidence of the median origin of the eye anlagen. Stockard's own diagrams (figs. 6 and 7, p. 275)[6] which are to prove that the eye anlage is primarily median in position would rather seem to support my arguments for the lateral origin of the latter, while his diagram in figure 8 (p. 276) most decidedly portrays a condition which is impossible, not, because the optic stalk tissue is (partly) of median origin, but because it is not a part of the eye vesicle.

From what has been said so far, it is obvious that Stockard's assumption of the single condition of the optic anlage and its median position in the medullary plate as a basis for the morphogenesis of synophthalmia and cyclopia is untenable.

From a study of my own abundant material in sections as well as from a careful scrutiny of the views presented by previous writers on the subject I have convinced myself that a *rational analysis of the morphogenesis of synophthalmia and synophthalmic cyclopia must be based on* Spemann's ('04, '12) and Lewis' ('09) *theory of a fusion of early* (pre-vesicular) *eye anlagen, due to a defect of intermediate tissue.* This conclusion I have reached in spite of the indisputable fact that the fusion theory is inadequate in the case of perfect cyclopia and that the nature of the defect that precedes the fusion has not yet been made quite clear.

In the following I shall attempt an analysis of the morphogenesis of teratophthalmia which is based on a recent physio-

[6] Owing to the great importance of the subject here discussed the reader is advised to consult Stockard's ('13) diagrams, without which this discussion may perhaps not be quite intelligible.

logical discovery of Child's as an important key to the understanding of the nature of chemical defects, and on the assumption of fusion of two originally separate early optic anlagen as underlying the formation of synophthalmic and cyclopean monstrosities.

2. The defect-theory of teratophthalmia

When Fundulus eggs are subjected to the influence of toxic solutions of a perceptibly injurious (but not lethal) concentration for a certain (not lethal) length of time, it can usually be found that among the many monstrous embryos which will develop, a certain rather large number will exhibit deformities of the eyes, while other parts of their bodies may appear not, or only very slightly, to deviate from the norm. In such embryos the eye deformities are often the most, if not the only, striking ones. Since the entire eggs were subjected to the influence of the toxic solution, it appears rather puzzling that the developmental product of the egg should show the effect of the treatment only at such a locally restricted area. In experiments in which mechanical methods (such as pricking—Lewis '09, or constriction—Spemann '04) are employed the local deformation can readily be accounted for by the locally restricted lesion which has been caused mechanically. In the chemical experiment, however, the treatment is not restricted to a part of the egg, and yet the effect is so often a restricted one. How can this be accounted for?

There is, so far as I am aware, only one known fact, which will account for this interesting phenomenon. This is Child's important discovery of the high susceptibility of the animal pole to noxious influences.

This author (Child '11, '12, '13, '14) has shown that if a ciliate infusorium or a planarian be subjected to the influence of lethal solutions of certain toxic substances the disintegration resulting in the death of the animal will proceed gradually from the anterior towards the posterior end of the body. The same results were obtained also in other adult invertebrates. In this manner a definite gradient of susceptibility was demonstrated to exist along the chief body axis.

Of quite particular interest in connection with my own results reported in this paper are Child's ('15 b) experiments on starfish eggs. If these were subjected at early stages of development to the influence of solutions of potassium cyanide not strong enough to kill the eggs, the resulting larvae exhibited the detrimental effect of the sojourn in the toxic solution mainly in the apical region.[7] Here, as well as in my experiments on Fundulus the part of the egg destroyed by chemical action is the one which corresponds potentially to the embryo's apical (animal) pole.

Very recently Child ('15 a) has concluded that this primary gradient from the 'animal pole' to the 'vegetative pole' is very general in organic life and that its demonstraton is only difficult in higher animals, where, owing to complex organization, the results of the 'resistance method,' by which they can be demonstrated in invertebrates, are obscured.

In the early embryo, however, before the differentiation of organs, when the physiological conditions are yet relatively simple, the assumption of such an 'axial gradient' in the susceptibility of the fish egg would seem to be justified.

If, however, this gradient of metabolic reactions exists in the fish egg, then there exists also a point of highest susceptibility and accordingly of least resistance; and this point (the animal pole) is the potential anterior end of the embryo's body. Granting this, however, it is no longer difficult to understand why the effect of the toxic solution on Fundulus eggs should, as it so often does, manifest itself in defects of organs of the anterior end of the body, and most particularly the eyes, the mouth, the olfactory pits, and the forebrain, while the rest of the body may suffer very little from the sojourn in the solution.

Many observations point to the conclusion that this injury at the apical pole which results in terata of the eyes, is caused mainly by a process of disintegration and dissociation which I have termed blastolysis. It is not easy to understand just what

[7] Very recently Painter ('15) observed that in Ascaris eggs which have by an accident come under the influence of carbon dioxide "roughly 33 per cent of the embryos (in 54 cases out of 165 examined for the point)" have sustained severe injuries at the anterior end.

chemical reactions may underlie this process. They obviously depend entirely on the chemical nature of the solution employed. Thus it would seem reasonable to expect that for instance they are quite different in butyric acid solutions from those of magnesium chloride solutions employed by Stockard ('09) or alkaloid solutions employed by McClendon ('12 b). The action of some solutions may dissolve, while that of other solutions may coagulate or precipitate certain substances of the egg. However, no matter what this action may be, it certainly results in a chemical alteration, which will be the more intense, the higher the concentration of the solution or the longer the time of exposure. Accordingly, if the action be a slight or moderate one, the chemical alteration may result only in an inhibition of certain groups of cells possessing a high degree of susceptibility, i.e. these cells may continue dividing and differentiating up to a certain point, beyond which, owing to exhaustion of their chemical capacity, they are unable to proceed. Or the action may be strong enough to cause, by chemical alteration, a check of the most important physiological processes (cell metabolism and cell division) of these embryonic cells, which would result in their disintegration. No matter what chemical solution be employed, if it only is injurious to life, it will in this way, have a destructive effect. If non-lethal concentrations and lengths of exposure be employed, this destructive process will largely be restricted to the animal pole of the egg, i.e., to that part of the egg which in normal development would correspond to a certain area at the anterior end of the potential embryo's body. Since, according to the rule of the 'axial gradient' this destructive process—chemical blastolysis—begins at the animal pole of the egg and cannot proceed further, owing to insufficient strength of the solution or to timely transfer to a normal environment (pure sea-water), its effect is eventually noted in deformities at the embryo's anterior end of the body.

According to what has been said so far the sequence of events leading to the deformities of the eye is, then, more or less the following.

A small part of the egg in the earliest stages of development corresponding to a restricted area at the anterior end of the future embryo's body sustains a chemical lesion, i.e., it becomes so altered chemically as to be incapable of the reactions necessary for its further normal development. The part of the egg thus incapacitated, is potentially the region anterior to and between the future optic anlagen or even the region of those anlagen. This affected area goes on developing up to a certain point beyond which, owing to the exhaustion of its chemical capacities, it loses its correlation with the whole, i.e., with the rest of the embryo-forming material and becomes eliminated by dissociation. Or, the affected area may, at that critical point of chemical incapacity, be permanently arrested and retain the characteristics of this early stage of development (some cases of anophthalmia), while the rest of the embryo may develop and differentiate further.

The size of this restricted area of blastolytic lesion at the anterior end of the potential embryo's body axis is probably subject to considerable variation. Thus it may comprise the mass of cells which would normally correspond to the future interocular area and cause an approximation of the potential optic anlagen or, it may extend over the latter ones and eliminate parts of them, while the uninjured parts would fuse after an approximation resulting from the healing of the wound, and form any one of the various degrees of the synophthalmic condition. Again, the injured region may comprise parts of the ophthalmoblastic material of both sides and very little of the potential interocular area. The remnants of the optic anlagen may develop and differentiate fully into eyes of strikingly small size of the microphthalmic monsters. Or, the lesion may comprise the whole of one optic anlage and little or no material of the future interocular area. In that case the embryo will develop into a perfectly cyclopean embryo, if, owing to subsequent regulation, the uninjured potential optic anlage is shifted medianwards, or into an asymmetrically monophthalmic monster, if no such change in the position of the uninjured or less injured ophthalmoblastic material takes place. Wherever in such cases

the sustained injury is of a still higher degree, i.e., if only a fragment of one optic anlage survives, the cyclopean or asymmetrically monophthalmic eye, formed from it, will be of a correspondingly small size. And, finally, if the entire ophthalmoblastic material is destroyed by the blastolytic process, the defect will result in anophthalmia vera.

At this point it should be noted that there is yet another kind of anophthalmia, which is not due to blastolytic destruction but apparently to an inhibition. In anophthalmic embryos of the latter category there are always found on microscopic examination eyes which owing to the exhaustion of their chemomorphic capacity, have remained at a very early stage of development (figs. 23 and 24, p. 494 and fig. 49, p. 520). This, however, is, I believe, the only instance where an ophthalmic deformity is due to inhibition, while all synophthalmic and one-eyed conditions owe their origin to a defect brought about by blastolytic elimination.

For a better understanding of the morphogenesis of ophthalmic terata it is also necessary to consider the time at which blastolysis brings about the changes leading to their formation. Since it can be shown that in Fundulus eggs under the influence of toxic solutions the chemical injury follows the rule of the 'axial gradient,' i.e., it proceeds from the anterior end posteriorwards, it seems safe to assume that the blastolytic lesion is sustained at a very early, primitive stage of development, long before differentiation of organs has yet begun.

In a previous paper (Werber '15 c, p. 558) I have advanced the view that the blastolytic injury is sustained before the formation of the embryonic shield. To be more precise, it may be said that this elimination of destroyed material most likely occurs at a late 'Randwulst'-stage of the germ-ring. For it is at this stage that very important events take place which eventually lead to the organization of the embryonic body and differentiation of tissues and organs. At that time of transformation of the blastoderm into the embryo which is accompanied by rather active movements or shiftings of the embryo-forming material, the contact between the sound, unaltered part of the

latter and the chemically destroyed one is loosened more and more until it is lost entirely and the altered fragment breaks off.

This will at once become apparent if we recall the facts regarding the formation of the embryo in teleosts so well established by the investigations of Kopsch ('96, '04). According to this author the formation of the embryo comes about in the following manner ('96, p. 120):

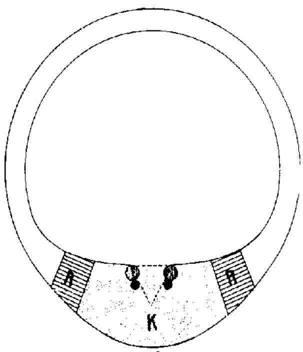
An dem zelligen Randring (der Keimscheibe 24 Stunden nach der Bildung des ersten Umschlages) muss man zwei Bezirke unterscheiden. Einen embryobildenden und einen nicht (direct) embryobildenden. An dem embryobildenden Bezirk, welcher an der Stelle der ersten Einstülpung gelegen ist, haben wir weiter zu unterscheiden einen der Medianlinie näher gelegenen Teil, dessen Zellen den Kopf des Embryos bilden, und jederseits lateral von diesem Bezirke Zellengruppen, welche im Laufe der Entwickelung in der Medianlinie zusammenkommen, und den Knopf bilden. Der Knopf stellt ein Wachstumcentrum vor, von welchem Rumpf und Schwanz gebildet werden, wobei Zellen des nicht direct zum Aufbau des Embryos verwendeten Teiles des Randringes im Laufe der Umwachsung des Dotters zum Knopf gelangen und dort ebenfalls zur Bildung des Embryos benutzt werden.

In both the area which is to form the head (K) and in the parts which are to form the 'Knopf' (figs. 50 and 51, p. 534) the organs of the head and trunk are potentially contained. For experiments of Kopsch have shown that if parts of any one of these areas be destroyed the organs which these eliminated fragments potentially represent, will be lacking (Kopsch '96, p. 121).

Fully in accord with these data is the view which I have expressed above, namely that terata of the head owe their origin to blastolytic injury at the anterior part of the area which is to form the head. This area would correspond to the area K in Kopsch's diagram and the blastolytic elimination of an antero-median chemically incapacitated fragment of this area must be assumed as taking place before the 'Knopf' has yet been formed, or thereabout. The eliminated fragment would contain parts of the material potentially representing maxillary and mandibular arches, the olfactory pits, the tissues of the interocular area and very often more or less of the ophthalmoblastic material. Fol-

lowing a suggestion made by Schwalbe and Josephy ('13, p. 205–206) I have attempted to portray this elimination diagrammatically, employing Kopsch's diagram as a basis (fig. 50).

In the dotted area K, which is the primordium of the head, the two horizontally barred circles represent groups of cells which would normally develop into the mouth and olfactory pits, while the two cross-barred circles immediately below represent the ophthalmoblastic material of both sides. If it now be imagined that blastolysis has eliminated a wedge of tissue which



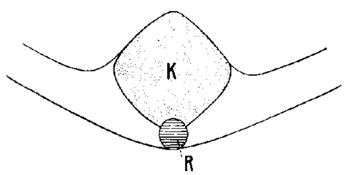


Fig. 50 Diagram of germ-ring with thickened embryo-formating area ('Keimrandwulst')—modified from Kopsch.

Fig. 51 Diagram of the early steps in the formation of the embryo from the thickened area of the germ-ring (from Kopsch).

as seen in the diagram, is directed with its point posteriorly, while it is broadest anteriorly, this wedge would contain, more or less, the potential interocular area, parts of the potential mouth and the potential olfactory pits of both sides. A considerable part of the potential forebrain would in this way also be eliminated. The gap thus resulting will soon close, owing to a contraction brought about by the elongation which accompanies the transformation of the head primordium of this stage into the head. What would be the final result of the elimination of such a wedge of tissue and the subsequent closure of the wound? A glance at figure 50 would show that the potential

anlagen of the mouth, olfactory pits and the eyes of both sides would in this way be brought into more or less close approximation or even into contact. Various degrees of the synophthalmic deformity would thus eventually result from the more or less complete union of both ophthalmoblastic areas. Incidentally the elimination of parts of the area potentially representing the mouth and olfactory pits will by fusion of their remnants lead to the formation of an elongated, more or less pointed mouth the 'proboscis' -and to fused olfactory pits.

A few words would now seem necessary regarding the size of the eliminated fragment and the distances between the respective organoblastic areas of both sides. While in our diagram, by which only a very rough portrayal of the presumable conditions is attempted, the eliminated area is relatively large and the respective organoblastic areas are pretty far apart, it is not at all meant to convey the idea that they are so in reality. Concluding from what appears to be lacking in the developed embryo and considering the fact that growth is an increase in volume, this destroyed anterior part of the head primordium must be conceived of as being relatively very small in size. It follows from these considerations that the bilateral ophthalmoblastic areas are at this stage relatively much nearer each other than they would be at a somewhat later stage, e.g., in the embryonic shield. Granting this, however, it is easy to account for the fact that the cyclopean eye is often much smaller in size than the normal eye. For, at this stage the eliminated area may often contain besides the potential interocular area a whole ophthalmoblastic area of one side and a part of the same area of the other side. The remnant of ophthalmoblastic material may develop laterally (monophthalmia asymmetrica) or into the median, genetically single eye of perfect cyclopia, if, owing to subsequent regulation it has come to occupy a median position. In the case of a small cyclopean, genetically synophthalmic (i.e., 'fused') eye it might be imagined that the destroyed 'wedge' of tissue contained a great deal of both ophthalmoblastic areas and that the remnants have, after fusion, developed into a median eye smaller in size than the normal eye. However, although I do

not deny the possibility of such small size in synophthalmic cyclopia, I would not leave unmentioned the fact that in my observations made on a great number of sectioned teratophthalmic embryos I have not yet had occasion to record a single such case. Examination of the figures in Stockard's ('09, '10 a) papers also very forcibly suggests that all cases of small cyclopean eyes which he pictures, represent perfect cyclopia, i.e. a condition of median, genetically single, one-eyedness, where a fragment of ophthalmoblastic material of one side developed into a small, but whole, eye in the median position, which it secondarily has come to occupy.

On the basis of our conception of the morphogenesis of ophthalmic terata, namely that the latter are due to a defect it is easy to account for the unquestionable fact that neither the perfectly cyclopean nor the synophthalmic eyes (cyclopia synophthalmica, synophthalmia unilentica et bilentica) are double the size of one normal eye, as Stockard ('13, p. 270) postulates they would have to be, if the fusion theory be regarded as correct. Such eyes simply cannot be "equal in mass to the two normal eyes fused" because the fusion in the synophthalmic eyes is due to destruction of a part of the eye forming material. This, however, meets an important and justified objection which Stockard has raised against the fusion theory of 'cyclopia.'

It is, no doubt, true that, while snyophthalmic deformities can be accounted for on the basis of this fusion theory, it would fail if extended to other ophthalmic terata. For, perfect cyclopia cannot, as was pointed out above, be regarded as due to fusion. Nor can it be denied that, as Stockard ('13, p. 278) remarks, the genesis of asymmetric monophthalmia and microphthalmia have yet to be accounted for. But while we admit these limitations of the fusion theory, it must be borne in mind that its most fundamental element—the exceedingly suggestive defect hypothesis of Lewis and Spemann will alone account for asymmetric monophthalmia and microphthalmia as well, as for perfect cyclopia.

The defect which in synophthalmia we have assumed to consist in a blastolytic elimination of a fragment of tissue from the

antero-median portion of the head primordium may, and, no doubt does, vary in quantity and location. The eliminated 'wedge' may involve an antero-lateral fragment only, which contains the ophthalmoblastic material of one side and in this case the embryo will lack one eye (asymmetric monophthalmia). Or, the 'wedge' may be a very broad and short one and involve parts of the ophthalmoblastic areas of both sides as well as a small antero-median portion. The result will be an embryo with small eyes, as a rule nearer each other than are normal eyes, and with deformities of the mouth (often proboscis-shaped) and the olfactory pits (microphthalmia).

Neither the unwarranted assumption of a single median eye anlage and an inhibition of its 'developmental vigor' (Stockard) nor, much less yet, degeneration due to deficiencies in the circulatory system as Loeb ('15) assumes, can be expected to solve the difficult problem of teratophthalmia. For, these deformities are clearly due to a defect of the undifferentiated embryonic primordium. With the possible exception of such cases of anophthalmia (in which on sectioning deeply buried rudimentary eyes are found) where an inhibition cannot be excluded, all eye malformations can be accounted for by a blastolytic defect. And the localization of the latter can, in turn, be understood, when we assume the presence of an antero-posterior axial susceptibility gradient, which Child's investigations have made so highly probable.

D. DEFORMITIES OF THE BRAIN

In nearly all pathological embryos recorded in our observations the central nervous system is more or less deformed. No detailed study of the malformations of this system has yet been made, and the few observations which will be recorded at this place pertain largely to the brain in teratophthalmic embryos.

In our description of ophthalmic deformities same malformations of the brain have already been pointed out (cf. pp. 503, 506, 508, 509, 511, 512, 513, 515 and 520). It was shown that in synophthalmic and cyclopean monsters the forebrain is single,

there being no division into a right and left lobe. This is evidenced very plainly by figure 52, which is a transverse section of a synophthalmic embryo, as well as by the cross sections of the cyclopean embryos illustrated in figures 63, 66 and 70. The mid-brain and hind-brain are mostly bilobed and otherwise symmetric. Sometimes, however, and usually only in cases of perfect cyclopia, the single unilobed condition may be found to obtain also in the mid-brain (cf. fig. 48, p. 513, also figs. 65 and 67) and even in the hind-brain (fig. 71).

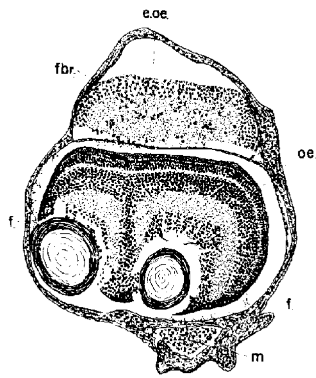


Fig. 52 Camera lucida drawing of a transverse section through the eye region of a synophthalmic embryo from acetone solution (35 cc. gram molec. to 50 cc. sea-water) thirty-two days old. *fbr.*, fore-brain; *oe.*, oedema; *e.oe.*, extracerebral oedema; *f.*, fibrin; *m*, mandible. × 100.

An important clue to the genesis of this disturbance in the symmetry of the forebrain (and rarely also of the mid-brain) is given by the size of the latter. In transverse sections it is often much smaller in area than the synophthalmic or cyclopean eye, as a glance at text-figures 48 and 52 and figures 63, 65, 66, 67 and 70 will convince. This indicates clearly that something is lacking in such brains. In other words, we are here dealing with defects. They are undoubtedly the same defects which resulted in the deformities of the eyes of these embryos, and are due to blastolytic elimination of a 'wedge' of tissue from the anterior

or anteromedian portion of the early, undifferentiated head primordium. In most embryos which exhibit no other than eye deformities, this destructive blastolysis affects the forebrain only, while occasionally the mid-brain, or a part of it, may to some extent be found to be involved in the process of destruction. Among many embryos which, while being teratophthalmic, appeared to be 'normal' in other respects, I have found one in which blastolytic fragmentation in the region of the mid-brain (one optic lobe) is very apparent. A transverse section at the level of the mid-brain of the embryo (fig. 13), whose one eye only was defective, the other being normal, is shown in figure 74. Examination of this figure shows that the left optic lobe (on the right side of the figure) is fragmented, the greater part of it being delaminated in such a manner that a wide furrow resulted between the two fragments of the lobe. The break through the optic lobe is, however, not complete, for if more posterior sections be examined, it can be seen that, at the basal (ventral) part, the lobe is unbroken. This finding, I think, is highly indicative of the blastolytic nature of the process which brings about the defects of the brain.

Our observations on the brains of teratophthalmic Fundulus embryos agree well with those made on the brains of human ophthalmic monsters. Here, too, the mid-brain and hind-brain usually (in cases where cyclopia is the only deformity present Ernst '09) are normal while the forebrain is small and unilobed. The single condition of the latter is so well known as to have even led some authors to the assumption that it is responsible for cyclopia. In our opinion it is syngenetic with the deformities of the eyes, the olfactory organ and the mouth, i.e., all these deformities are genetically due to the same defect - the blastolytic destruction of a certain (more or less wedge-shaped) part at the anterior end of the early head anlage.

The chemical alteration which underlies the blastolytic lesion is, as has been pointed out repeatedly in the preceding, the greatest most anteriorly and diminishes posteriorwards along a line corresponding with the future embryo's chief body axis. Most anteriorly this alteration leads to destruction and elimina-

tion, while decreasing posteriorwards, it becomes gradually less and less destructive and only so interferes with the typical physiological reactions, as to diminish the capacity for normal differentiation. Thus in more posterior regions of the head of some teratophthalmic embryos the result is not an elimination of tissue but often a developmental inhibition.

Such an inhibition manifests itself mainly in the cells of posterior parts of the brain. They sometimes remain in the neuroblast stage in spite of the relatively advanced age of the embryo. This can be observed particularly in embryos in which the malformation has involved more than the eyes only.

Another observation which points to inhibition is that concerning the presence, usually in the mid-brain and more posterior parts of the brain, of many large tissue spaces (fig. 69), which are sometimes clear and empty while often they may be filled with fibrin (figs. 45, 46 and 48). These spaces evidently represent persistent early vascular anlagen which, owing to chemical incapacity, have been unable to develop any further and connect up into continuous vascular channels. The probability of the latter assumption is much enhanced by the fact that the heads of such embryos during life, owing to accumulation of fluid in the tissue spaces, are sometimes greatly distended.

This intracerebral oedema in the fish embryo, resulting apparently from the absence of a continuous system of haemal and lymphatic drainage, is, since the fish brain has neither lateral ventricles nor a choroid plexus, in a manner comparable to the congenital internal hydrocephalus of man. Such a condition of hydrops is often found to exist also in the cranial cavity which in these cases is unusually distended and sometimes contains fibrin (cf. figs. 46, 48 and 52). The extracerebral oedema is also probably due to blood or lymph vascular imperfections resulting from a developmental arrest. It is comparable to the human congenital external hydrocephalus, i.e., a condition, where an accumulation of fluid is found in the arachnoidal space. Both the extracerebral and the intracerebral oedema may sometimes be found in the same embryo (figs. 46 and 48).

The suggestion would thus seem to be at hand that both forms of hydrocephalus in man may be due to local inhibition

of the development of the blood and lymph vessels in the head region and the resulting lack of adequate drainage. Here, however, much further study is necessary. Careful anatomical investigations of well diagnosed cases of both forms of congenital hydrocephalus in man may possibly bear out the validity of our assumption. Much might be gained also from studies of the blood and lymph vessels of oedematous regions in experimentally deformed Fundulus embryos.

E. THE MICROSCOPIC ANALYSIS OF SOME TERATOMATA (THE 'SOLITARY EYE' AND THE 'ISOLATED EYE')

The tendency of Fundulus eggs when treated with butyric acid and acetone solutions, to give rise to partial embryos (meroplasts) has been noted in a preceding chapter (p. 499). It is obvious that a careful anatomical study of these monsters is very desirable, for their internal organization often discloses conditions of great ontomechanical significance. At the present time, while this study is yet incomplete, it may suffice to state that from microscopic examination of such embryos one gains the impression that the entire embryo-forming material has suffered greatly from blastolysis and that after a haphazard reconstitution of the surviving, but disarranged, parts of the germ the latter have developed into an embryo which is neither a whole, nor a symmetrical part of a whole. True anterior hemiembryos, i.e. formations in which an anterior half of the body (e.g. beyond the level of the pectoral fins) is present, while the rest of the body is lacking, have also been found. However, such cases are relatively rare. For, usually the blastolytic injury, whenever it is of a high degree, affects, to a greater or less extent, the whole embryo-forming substance.

Thus, it may happen that nearly all of the embryo-forming material suffers destruction and that only a very small fragment of it survives, which, however, is capable of further independent —development and differentiation.

As the most interesting cases of this category may be regarded some eggs in which all that can be found on the yolk-sac (excepting some rudimentary bloodvessels) is a fragment of tissue with

an eye (figs. 38–41) which may be single or composite (synophthalmia, fig. 39). In the fragment of nervous tissue from which such an eye arises the form relations of a brain do not exist, nor is it possible to recognize what parts of the brain are represented by the fragment.

One of such eggs with a 'solitary eye' has been briefly described in a previous paper (Werber '15 c). The significance of such formations being obvious, it seems desirable to give at this time a fuller description of it as well, as of two more eggs with solitary eyes which have been studied in sections.

In figure 40 is seen an egg without an embryo but with several tissue fragments on the yolk, distant from each other, one of which has given rise to a small eye discernible in toto.

The sections into which this egg has been cut, run parallel with the vertical axis of the solitary eye. Examination of the entire series (only one section missing) discloses the following conditions.

Nothing can be found in the yolk in any of the sections. Thus the possibility is entirely eliminated that the embryo had sunken into the yolk leaving one of its eyes (constricted off) on the surface. Several blood lacunae can be seen on the yolk-sac, some filled with erythrocytes, while others are densely packed with large cells of the appearance of leucocytes. The sections through the tissue fragment from which the eye arose, present a view somewhat similar to a transverse section of a rudimentary spinal cord (fig. 53). In further sections, proximal to the eye, this fragment of nervous tissue increases in volume so much (fig. 54) that it seems safe to regard it as a—malformed, highly defective—brain. Partially in contact with the latter is the lens, which appears in further sections, after part of the optic cup has already come into view. The whole eye is very small and is rather deeply imbedded in the yolk (fig. 55). Of its component parts the retina, and the lens are best developed. No iris is present, and the cells of the cornea are poorly differentiated. The space relations of the eye to the 'brain' which has given rise to it are much distorted, the brain enclosing part of the eye.

Of the other tissue fragments, which have been observed in the living egg, as well as after fixation, one has proved to be another solitary eye. This other eye is also seen to arise from nervous tissue, which, obviously, is a (very defective) brain (fig. 56). The retina of this second solitary eye is in a very rudimentary stage of differentiation, no pigment layer is present, nor an iris; the cornea is very poorly differentiated, while the lens is practically perfect. This second eye is much smaller than the first one described, from which it is 262μ distant.

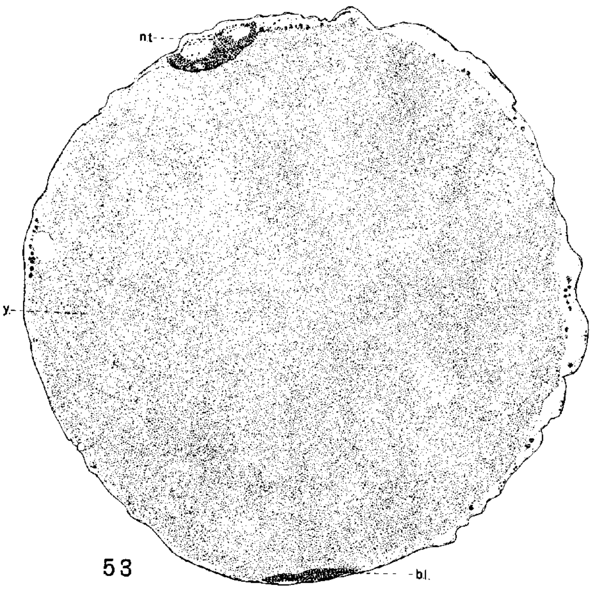
These microscopic findings are very significant for the analysis of the morphogenesis of the solitary eyes of this egg. Since no embryo can be found in the egg, it is obvious that with the exception of very small fragments all of the earliest embryonic primordium has suffered destruction. It is likewise obvious from the great distance between the surviving fragments which have given rise to solitary eyes that they have secondarily been shifted to distant parts of the yolk. The destruction of the embryonic anlage is possibly in the nature of a blastolytic disintegration (chemical blastolysis), while the apparent dispersion of its small surviving remnants would seem to be due to an increase in the eggs internal osmotic (exosmotic) pressure (osmotic blastolysis).

That both chemical (toxic) and osmotic blastolysis underlie the origin of such meroplastic formations is indicated by the next case of 'solitary eye' now to be described.

On examination in toto (fig. 40) of the transparent egg (both while living and after preservation) no embryo could be seen anywhere. All that could be found on the yolk was a small meroplast, which made the impression of a very large eye with a protruding, unproportionally large lens.

The true conditions obtaining in this meroplast, however, are somewhat different. For, examination of sections proves unmistakably that this meroplastic formation presents a case of solitary synophthalmia.

In the first three sections (fig. 57) there appears the lens of one of the eye components (left). It is perfectly well developed and surrounded by an epithelial capsule. On one side of the cross-sectioned lens is seen a thickened mass of tissue which



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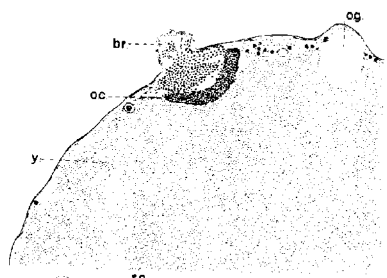

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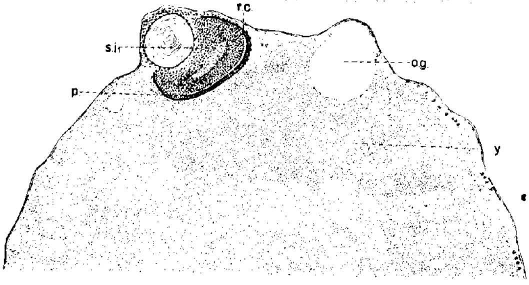

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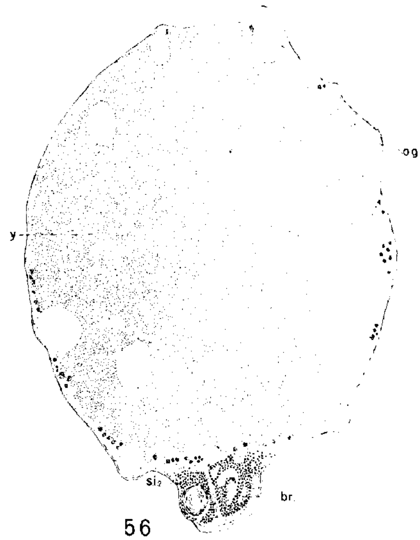

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Figs. 53 and 54 Camera lucida drawings of sections through egg in figure 40, *n.t.*, nervous tissue fragment, (brain, *br.*), from which solitary eye arose. *o.c.*, optic cup; *b.l.*, blood lacunae; *y.*, yolk; *o.g.*, oil globule. × 78.

Figs. 55 and 56 Camera lucida drawings of sections posterior to those in figures 53 and 54. *s.e₁.*, first eye-teratoma ('solitary eye'); *r.c.*, rods and cones; *p.*, pigment epithelium; *s.i₂* second 'solitary eye;' *br.*, brain; *y.*, yolk; *o.g.*, oil globules. × 78.

Figs. 57 and 58 Camera lucida drawings of sections through egg with eye-teratoma ('solitary synophthalmia') in figure 39, *l.*, lens; *l.c.*, lymphocytes; *pl.*, plasma; *br.*, brain fragments; *p.*, pigment epithelium; *n.*, optic nerve; *y.*, yolk; *o.g.*, oil globule. × 78.

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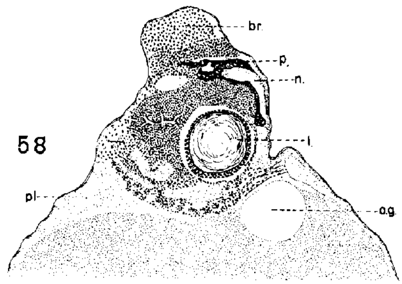

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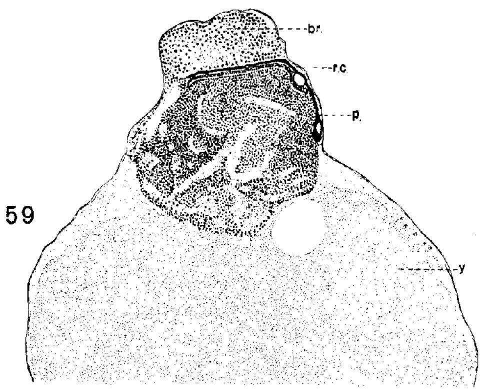

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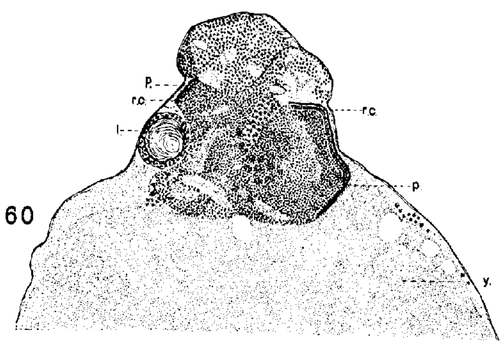


Figs. 59 and 60 Camera lucida drawings of sections posterior to those in figures 57 and 58. *br.*, brain; *r.c.*, rods and cones; *p.* pigment epithelium. *l.*, lens. × 78.

makes the impression of an ill-differentiated cornea. Between the lens and the yolk there is to be seen a very wide space filled with a plasma-like mass and lymphocytes. In farther sections an optic cup and still further a small unilobed, oedematous brain comes into view (fig. 58). A part of the pigment epithelium is also seen in this section; it partly surrounds a nerve trunk (apparently the optic nerve?), whose course is a very tortuous one. In further sections posterior to the lens the rods-and-cones-layer of the retina can be easily recognized (fig. 59), while the other layers seem to be ill-differentiated and (owing to blastolysis) considerably intermingled.

In still further sections through the meroplast the lens and the optic cup of the second eye component gradually come into view (fig. 60). The optic cups of both eye components are fused for the most part and if the whole series of sections be followed out, only the bottoms of the optic cups appear to be separate. The analogy between the morphology of this case of solitary synophthalmia and that of bilentic synophthalmia described in a preceding chapter of this paper (cf. p. 503) is evident.

What is the morphogenesis of this meroplast? The fusion of two early undifferentiated eye anlagen has evidently occurred after chemical blastolysis has eliminated a wedge of intermediate tissue. This blastolytic process has probably taken place while the egg was in the toxic solution, while on subsequent transfer to pure sea-water owing to increased imbibition of sea-water, resulting from the treatment of the eggs with acids, osmotic pressure has apparently destroyed all of the embryonic primordium excepting the small part which has given rise to the synophthalmic head fragment.

One more egg with a solitary eye may yet be briefly described. In figure 38 which illustrates it, there can be seen a rather large, tissue fragment with a very large eye. Close to it is a large oedematous (pericardial?) vesicle. Some small blood vessels have also developed, but there is neither a heart nor a continuous system of circulation. Owing to the considerable size of the tissue fragment from which the eye arises, as well as to its

peculiar shape, the interpretation of the case in toto appeared difficult.

In sagittal sections, however, it was recognized that the large size of the meroplast was due to oedema. In figure 81 is presented such a section which is near the longitudinal midline of the meroplast. The view is that of a transverse section of a rather distorted diencephalon and a large, longitudinally sectioned eye. The latter is well developed; the pigment layer is rather delicate, the rods-and-cones-layer of the retina, which is considerably distorted, is well differentiated, as is also the lens. Between the pigment epithelium and the other retinal layers of a large part of the eye there is to be seen an oedematous space filled with plasma and lymphocytes. This oedematous condition is responsible for the unusually large size of the eye, as well as for the distortion of the retina. For, in further sections, where the oedematous space gradually disappears, the wall of the optic cup has a practically normal appearance. Both the eye and the brain are situated between the yolk-sac and the yolk. The brain, while very close to the eye, is partitioned off from the latter by an ectodermal lamella. It thus appears to be almost enclosed in a separate cavity which is surrounded by the blastoderm on all sides but one. This cavity is, however, not entirely taken up by the brain, for as seen in figure 81, it contains several large empty spaces filled with either plasma or leucocytes. These findings explain the large size of the meroplast as well as the peculiar shape of the tissue fragment from which the eye has arisen.

The three cases of solitary eye described above furnish ample evidence for the fact that a fragment of the medullary plate may be capable of independent development to such a high degree as to give rise to a well differentiated eye.

A somewhat similar case has been recently reported by J. Loeb ('15, p. 96). In the monstrosity which he produced from a Fundulus egg by low temperature "one eye with a lens and the tail was all that could be recognized". (cf. Loeb's fig. 13). His interpretation is to the effect that "the whole embryo had developed but the eye had survived while the other parts perished."

Something, no doubt, did perish in the egg which Loeb describes but it did so before the formation of the embryo. This early death of the part of the germ which was to form the part lacking in the described egg is due to disintegration caused by the noxiously low temperature. The parts of the germ which have survived have eventually developed—independently—into a head fragment with an eye, a clubby tail and a heart. The analogy of this case with the 'solitary eye'-formations recorded in my work is obvious.

That such cases are not due, as Loeb suggests, to the death of a part of the already formed embryo is further evidenced very strongly by some eggs in which besides a complete, but teratophthalmic embryo, there has developed independently and at a great distance from the latter a small tissue fragment with an eye. In the course of the experiments reported in this paper three such eggs with "isolated eyes" have been recorded[8] (cf. figs. 29, 42 and 43).

One of these eggs (presented in figure 42) may now be briefly described. The embryo is asymmetrically monophthalmic and is also seen to lack all fins. No other defects could be observed in toto. On the yolk-sac very near the distal end of the unusually large pericardial vesicle a small round tissue fragment can be seen. Below it and at a very great distance from the embryo a much larger tissue fragment with a well developed eye can be observed.

On microscopic examination of sections of the entire egg it could be recognized that the smaller tissue fragment is a rudimentary eye and that the interpretation of the larger tissue fragment as an isolated eye is perfectly correct.

The egg was cut into sections running transversely through the embryo. In the most anterior sections through the embryo (fig. 82) there can be seen on one side of the yolk the malformed brain of the embryo with the anterior part of the eye, while on the yolk's opposite side the smaller one of the isolated fragments comes into view. This tissue fragment can, on careful examina-

[8] More recent experiments (not yet published) have yielded a relatively great number of eye-teratomata of both kinds (with and without an embryo).

tion, be recognized as a very rudimentary optic cup with an imperfectly differentiated lens. The latter is (as can be made out without difficulty even at the low magnification of the figure) in a stage transitional between the cellular of the lens-bud and fibrillar of the fully developed lens. In sections more posteriorly, and beyond the level of this first isolated eye, the second and larger one comes into view exactly opposite the monophthalmic embryo's brain (fig. 83). Two small lenses are first seen in this eye, the retina of which has an early embryonic, rudimentary appearance. In more posterior sections, the smaller of the two lenses disappears, while the larger one appears to be of a size to fit the eye, in which at this level the retina appears to be better differentiated, its rods-and-cones layer being clearly discernible (fig. 84). In still further sections posterior to this larger isolated eye there comes into view the highly malformed brain fragment from which the eye has arisen.

There is no connection whatsoever between these two isolated eyes and the embryo. Like conditions obtain in the two other eggs with isolated eyes (figs. 29 and 43) which have also been examined in sections.

No degeneration, as suggested by Loeb, can account for such cases. For, there are no processes of degeneration to which the already formed embryo might be subject other than (occasionally) necrotic (cytolytic?) changes. The latter, however, lead to the entire embryo's rapid death.

These teratomata present one more striking proof for the correctness of the assumption that the injuries sustained by the eggs, due to a noxious alteration of the environment, are of a blastolytic nature. The 'isolated eye' as well as the 'solitary eye' develops from a fragment of the anterior end of the potential embryo's body. In the first case the remainder of the germ's substance survives and develops into a teratophthalmic embryo, while in the latter the fragment which eventually develops into an eye is all that survives of the germ. These effects are due mainly to two causes, namely to chemical alteration of the germ's substance which is greatest at the anterior end of the future embryo's body, and to increased osmotic (exosmotic) pressure

on transfer to sea-water. The degree of resistance to the latter is the lowest at the point where the chemical alteration has been the greatest. Owing to this circumstance, apparently, it is from this part of the germ's substance that small fragments become occasionally split off by the pressure and shifted away from the potential embryo's position on the yolk. The increased osmotic pressure probably destroys entirely some eggs, for shortly after transfer of the surviving eggs to pure sea-water a number of them can usually be found to be dead. In some instances, however, small parts of such an egg which suffered great destruction from osmotic pressure may survive. This fragment, occasionally, by its differentiation into a solitary eye betrays its origin from the anterior portion of the potential medullary plate.

In commenting on the case which he reports, Loeb (l.c.) remarks that "the analogy with teratomata is obvious." Regarding this point I fully agree with Loeb, although teratomata could not by any means owe their origin to degeneration of an already formed embryo. They develop from fragments of the embryonic primordium which has sustained (blastolytic) injuries before the differentiation of tissues and organs. This point I have very recently ascertained by observation on living eggs, and I can fully confirm the correctness of Roux's ('95) generalizations on the subject. The latter author discussing the significance of some monsters recorded in the teratological literature arrives at the following conclusions (p. 205):

> Die Ursachen dieser hochgradigen Defect-und auch zum Theil der grossen Deformationsmissbildungen wirken, so viel wir jetzt sehen, schon zu Zeiten, ehe die einzelnen Organe und Gewebe vollkommen angelegt oder ausgebildet sind; daraus folgt, dass *die betreffenden Theile des Embryo 'nach' so hochgradigen Störungen ihrer selbst oder ihrer Nachbarschaft* nicht blos noch am Leben zu bleiben, sodern sogar *in einer oft noch an das Normale erinnernden Weise sich formal und geweblich zu differenziren vermögen.*

And further on, commenting on the capacity of small parts of an embryo to develop and to differentiate, Roux thus says (p. 207):

> Wir ersehen aus den angeführten Beispielen, dass *viele 'Theile' des Embryo unter günstigen Ernährungsumständen sich unabhängig von ihrer*

näheren oder ferneren Umgebung geweblich und formal zu differenziren vermögen, und dass dies sogar in annähernd normaler Weise geschehen kann. Daraus geht hervor, *dass die Differenzierung dieser Theile an sich nicht eine Function der Wechselwirkung zwischen ihnen und den andern Theilen ist.*

These views of Roux apply very well to a great many teratomata which I have recorded, and among them the 'solitary eye' and the 'isolated eye' as well as to the case described by Loeb, to which I have referred above.

The 'solitary eye' and the 'isolated eye' are experimentally produced teratomata analogous to some well known spontaneous teratomata (e.g., 'dermoid cysts') in which hairs, skin, glands, and jaw fragments with well formed teeth can be found. According to Schwalbe ('07, part II, p. 99) eye parts have been repeatedly found in teratomata. Both Roux and Schwalbe regard such formations as striking evidence for the great capacity for self-differentiation possessed by parts of the embryo. In the light of this well supported view the morphogenesis of teratomata loses all of the mystery which has so long surrounded it. It is certainly not more difficult to comprehend than the differentiation of neurones from a minute fragment of medullary plate tissue explanted in Harrison's ('07, '10) pioneer experiment into a favorable nutritive medium (lymph). In both cases fragments of a primordium survive and differentiate fully, if nutritive and other conditions necessary for the continuation of life remain essentially unaltered.

It follows from what has been said that the morphogenesis of some teratomata ('dermoid cysts,' 'solitary eye,' 'isolated eye') can be accounted for only by a very high ability for 'self-differentiation' of metablastolytic fragments of the embryonic primordium. The conclusion is also evident that at a very early stage of development (coincident probably with the teratogenetic time limit) the parts of the embryonic primordium are already very sharply predetermined in regard to their histogenetic and organogenetic potencies.

F. THE MICROSCOPIC ANATOMY OF SOME AMORPHOUS MONSTERS

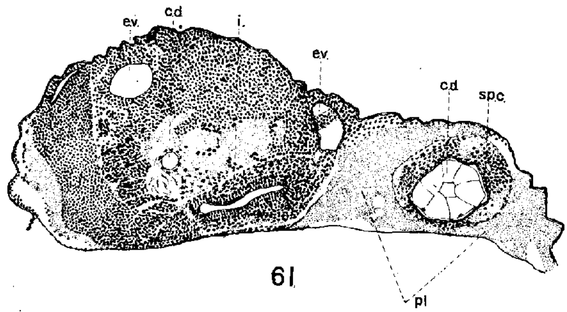
As has been stated in a preceding chapter (cf. p. 498) these products of pathological development, due to treatment of eggs with butyric acid or acetone, resemble, when examined in toto, very much the amorphous monsters of man. Schwalbe ('07) has already pointed out that anatomical examination of the latter, often discloses conditions which are of interest from the onto-mechanical point of view. Bearing in mind the relative rarity of the spontaneous occurrence of such monsters, an anatomical investigation of the amorphous monsters recorded in my observations would appear to be desirable. My study of these embryos, however, being as yet incomplete, I shall confine myself for the present time to the following description of three such monsters with a view of returning to the subject in a later publication.

In figure 44 is seen a very misshapen embryo which makes the impression of coalesced, greatly malformed twins.

Examination of the entire series of sections, however, proves that the appearance of the embryo in toto is grossly misleading. For the case is not one of coalescence of two embryos, but rather of a peculiar disarrangement and distortion of parts of one embryo's body.

In the first sections there appears the deformed head of an embryo and a transversely cut tail in which the notochord only can be distinctly recognized. Between the head and the tail and partly under the head there is between the yolk and yolk-sac a space which is filled with a plasma-like mass. The brain of the embryo is greatly deformed and unilobed. One of the eyes has developed in a rather rudimentary manner; its retina and lens, however, are well differentiated. The other eye is lacking. Only a well developed lens can be seen on this side, and in some sections the neighboring nervous tissue, arranged in a cup-like manner around the lens, exhibits some resemblance to a retina in the earliest stages of differentiation. At this level there can be seen in the adjacent cross-sectioned tail, besides the notochord, also transversely cut coils of the intes-

tine. No spinal cord, however, is yet seen in these sections of the tail, while in more posterior sections the notochord of the tail-part appears to be practically surrounded by the spinal cord (fig. 61). At the same level parts of the semi-circular canals



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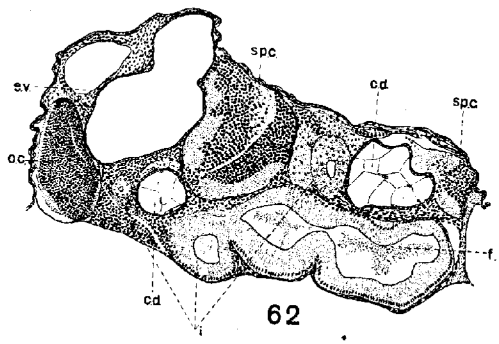

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Figs. 61 and 62 Camera lucida drawings of embryo in figure 44. *e.v.*, ear vesicles; *br.*, brain; *sp.c.*, spinal cord; *c.d.*, notochord; *o.c.*, optic cup; *i.*, intestine; *pl.*, plasma; *f.*, fibrin. × 100.

come into view and also a part of an obliquely cut gut-coil. A few sections more posteriorly the plasma-filled space between the two parts of the embryo has disappeared from view and its place is now taken up largely by the obliquely sectioned intestine which stretches across between the two parts. The latter are now continuous. The spinal cord of the tail-part is now restricted to the region dorsal to the notochord and can be

observed to gradually assume a shape approaching the normal. At this level one of the ear vesicles has disappeared from view while that of the other side (a very large one) is very prominent in the section. Laterally and ventrally from this ear vesicle (fig. 62) is seen a rudimentary optic cup. In the last sections the spinal cord runs across between the two parts of the embryo. It is particularly in these sections that the true nature of the monster is disclosed. From them we see that the posterior half of the embryo's body is in relation to the embryo's longitudinal axis turned at an angle of almost 180°. An oedematous blastodermic cavity filled with plasma and extending between the two so disarranged parts of the embryo has given the latter the appearance in toto of coalesced very malformed twins.

What is, now, the probable morphogenesis of this monster? The presence of one eye only would, according to what has been stated in the preceding, point to blastolytic injury sustained by the early embryonic primordium. This injury seems to be evidenced by the following facts. The ophthalmoblastic material of one side has, owing to chemical alteration, been somewhat inhibited in development and has given rise to an imperfect eye. The ophthalmoblastic cells of the other side have suffered great destruction (owing to increased osmotic pressure after chemical alteration?) and a remnant of them has been fragmented into two parts. Of the latter one has made some initial steps in development (having attained the shape and to some degree the structure of an optic cup) and stimulated the development of a lens, while the other fragment has been dislocated and developed into a rudimentary optic cup at the posterior level of the ear vesicles. The latter are both deformed and oedematous. The deformities of the brain are such as to point to a hap-hazard regulation of a brain primordium after blastolytic lesion. The oedematous blastodermic cavity filled with plasma suggests that no continuous blood circulation existed, the failure of blood vessels to develop being due to chemical alteration—a condition which can be found nearly always in embryos which have sustained blastolytic injury of a high degree. The strange space relation of the posterior half of the body to the anterior would apparently indicate the action of osmotic pressure, which

while not sufficiently great to cause a rupture of the two halves of the primordium, has only shifted one of them out of its normal position on the yolk. Briefly, it may be said, that the effect of blastolysis, due to chemical alteration combined with osmotic pressure, was in this case a highly deformed amorphous embryo, which as figure 44 shows, can in toto easily be mistaken for coalesced malformed twins.

To blastolysis by chemical alteration and increased osmotic pressure are evidently due also the conditions found in the following embryo now to be described.

As can be seen from figure 30 the embryo is very deformed and only slightly suggests the body form of a fish. The irregularity of form pertains particularly to the head and the trunk, which latter appears to be greatly distended. There is no clear indication of the true nature of the embryo, namely that it is, as examination of sections shows, a case of a partial, fused duplicity.

The most anterior (transverse) sections show an anophthalmic and sharply pointed, highly oedematous head. Between the head and the yolk-sac there is a space filled with plasma (fig. 85) which can be recognized as the enlarged, oedematous pericardial vesicle. A lens is seen situated laterally, but there is no trace of an optic cup. Practically at the same level with the lens there appears ventro-laterally to it a transversely sectioned tube-like structure (gut?) which can be followed only in eight sections of 7μ thickness. Following the sections posteriorwards the oedematous brain is seen to increase in size and to be highly deformed. Still more posteriorly, i.e. at the posterior level of the lens the brain mass makes the impression of two fused, malformed brains, there being between the components a distinct lamella.

In sections through this double brain mass there can be seen at a distance from the embryo a transversely cut fragment of nervous tissue which makes the impression of a deformed spinal cord. It corresponds to the tissue fragment (*tf.*,) in figure 30. Between this fragment, the embryo and the yolk, there is a cavity filled with a plasma-like mass (fig. 86).

A second tissue fragment (tf_2 of figure 30) can be seen in more posterior sections on the side of the yolk opposite the embryo. This fragment proves on careful examination to be a very rudimentary eye, of which only the lens can be recognized with certainty.

The larger one of the two brain components (a) is more oedematous and its staining reaction with haematoxylin is weaker than that of the other component (b). This fusion of two brains into one mass becomes very distinct in still more posterior sections where component a now gradually diminishes in size, until it entirely disappears from view while the component b has encroached upon its place in the section. Several sections further posteriorly (fig. 86) there is seen a large optic cup at the ventral part of the brain component b. Retinal cells (rods and cones) of this optic cup can be recognized in some sections. The following is the complete picture presented at this level. Almost half of the section through the embryo is taken up by brain component b. Laterally from it is seen a transversely cut notochord, while ventral to it is the optic cup, and ventrally from the latter are seen cross-sectioned coils of an intestine. In the intestines a fibrin-like mass can be noticed, which points to defects in the circulatory system. Following the series more posteriorly there can be seen gradually to appear the spinal cord above the notochord. It is at first on one side fused with brain component b while in sections at a more posterior level (fig. 87) it is separated from the latter by a mass of mesodermal cells (poorly differentiated myotomes?). In still further sections the brain component b disappears entirely while a large cavity lined with endothelial cells and filled with plasma is seen to follow it in the sections. The last sections are cross sections of a deformed tail.

At the outset of the description of this monster it has been stated that the conditions found on microscopic examination point to blastolysis as a factor underlying its formation. While of course, a complete analysis of the morphogenesis of this monster is practically impossible, the two extra-embryonic tissue fragments would seem to point decidedly to blastolytic action. Considerable difficulty, however, is presented by the interpre-

tation of the double, fused brain. Here an additional assumption may be made, namely that fragments of the blastolyzed embryonic primordium have secondarily come to fuse owing to shifting caused by increased osmotic pressure. Such spatial distortions and shiftings can be recognized in many duplicities. In our present case this hap-hazard regulation and fusion of parts would seem to be evidenced by the unusual position of an optic cup on the ventral side of the posterior part of the brain (cf. fig. 86).

That amorphous monsters result from blastolytic injury of a high degree is well illustrated also by the anophthalmic and greatly malformed embryo in figure 31.

Besides the defects and malformations which are seen on examination of the embryo in toto more are disclosed when sections are examined. There is no trace of eyes, and the nervous system is lacking almost entirely. In transverse sections (fig. 88) through the region of the head only some muscles, connective tissue, cartilage (of maxillary or mandibular arches?) and very rudimentary, closely approximated ear vesicles can be seen. In a few, more posterior sections there can be seen what might be taken for a very faint suggestion of nervous tissue in a very poorly differentiated stage. It is evidently this exceedingly small remnant (only seven sections of 7μ thickness) of the nervous system that has given rise to sensory part of the vestigial ear vesicles. Somewhat further posteriorly transversely sectioned coils of the intestine come into view. No trace can be found of either the notochord or the spinal cord. The fins, which also come into view at this level, are oedematous and filled with plasma (fig. 89). A few sections further posteriorly there is seen ventrally from the intestine a large coelomic cavity containing some fibrin. This cavity enclosed by the body wall is practically all that can be seen in the last sections of the monster.

IV. CONCLUDING REMARKS

It has been shown in the foregoing that an almost endless variety of monstrosities can be produced in fish, if eggs in the very earliest stages of development are subjected to the action

of some toxic products of pathologic metabolism. The monstrosities thus produced resemble very much those occurring spontaneously in man and in other mammals. In other words, effects of unknown processes occurring in nature have been practically duplicated on a very large scale by the laboratory experiment.

In the latter the initial cause is known, because controlled by the experimenter. Some further steps in the sequence of events can, as I have recently convinced myself, partly be observed, and partly deduced from anatomical examinations of great numbers of monsters. The results of the latter agree well with those of anatomical examinations of the spontaneous monsters of mammals. Provided that in nature the same or very similar causes are responsible for the origin of monsters, the whole problem of teratogenesis in nature is—theoretically at least within the control of the experimenter.

On the other hand, the experimental results recorded by previous work in embryology and teratology all seem to point to the conclusion that of teratogenic factors there are many, indeed almost as many probably as there may be agents injurious to organic existence. On what basis then, may it properly be asked, can experiments be performed with the aim of control of the causes, if the latter ones can only be a few out of a very great number of possible ones?

The problem, while obviously beset with many difficulties, is, however, not quite as elusive as it would at first appear. For, while there is probably an endless number of factors which, if acting on the egg, might cause it to develop in an atypical manner, it is, for our purposes, necessary to consider only those which can reasonably be conceived as acting in the mammalian body under certain unusual conditions. The latter are, as we shall presently see, relatively few.

These factors must be either of a physical or of a chemical nature.

Of the physical factors which might interfere with the typical development in utero of mammals only pressure and an increase in temperature could be imagined. The former has, indeed, for a long time been regarded as the cause underlying the origin of monsters. The inadequacy of this mechanical theory of terato-

genesis has been repeatedly pointed out, and notably by Mall (l.c.). Neither is it probable that such an increase in the temperature of a mammalian female as would yet allow it to survive could materially affect an ovum in utero.

If, after what has been said, we dismiss the possibility of physical factors as the underlying causes of atypical development, there would remain only the group of chemical factors. Of the latter the number of those which can reasonably be imagined to be present in the mammalian's body under certain conditions, is rather limited. They might be some extraneous poisonous substances which may chance to gain entrance into the human (or other mammalian) body, they could also be bacterial toxines, or, finally, they may be autogenous poisons of the body, such as some toxic products of a disturbed metabolism.

Of the extraneous poisons which may find a way into the body without killing the individual, but only exerting an injurious influence on it, or on a developing ovum contained in the latter's uterus, there are probably not many. Alcohol and some other drugs, to which individuals may habitually be addicted, would probably occupy an important place among these substances. Lead-poisoning and phosphorus-poisoning might perhaps also be considered under this heading. However, while in the light of recent data, there is hardly any doubt left regarding the deleterious effect of parental alcoholism on the offspring (cf. Stockard '12) *no cases of human monsters are yet known of an alcoholic parentage.* The same would apply also to the occupational diseases of lead-poisoning and phosphor-poisoning. It cannot be denied that these poisons probably are capable of altering the typical course of embryonic development, but, evidently, they seem to be acting in a degree not sufficient to cause the development to become monstrous. Or, possibly the strength of action of these poisons necessary to produce monsters might kill the parent or bring about its sterility, and thus, no occasion may exist for human ova to be under the influence of such strong action of these poisons as would very materially alter the course of their development. The conspicuous lack of data regarding the relation between alcoholism and 'industrial diseases' on the one

hand and the occurrence of monsters in man on the other hand would seem to point to the correctness of our conclusions. Besides, many monsters similar to human monsters are found in other mammals (and in sauropsids) which are neither exposed to the dangers of alcoholism nor to those of 'industrial diseases.' These considerations have led me to believe that poisons such as alcohol and other drugs as well as poisons of the 'occupational diseases' only very rarely, if ever, may lead to monstrous development in nature. The percentage of monsters whose origin may be due to these causes must, at best, be regarded as so small, as to be practically negligible.

Let us now turn to the next known chemical modification of the mammalian body, namely to the toxines of infectious diseases. Is there any likelihood that such toxines, when present in the blood of a female, would have such a deleterious effect on the ovum in its uterus, as to seriously derange the course of its development? This question is as yet difficult to answer, for the existing data on the subject are too insufficient and too indefinite. But even if future researches should prove the probability of a deleterious effect of maternal infectious diseases on the embryo in utero, which at present seems doubtful, there would remain only one other great source of chemical alteration of the ovum's environment, namely the products of pathologic metabolism. Of the latter there is a considerable number known at the present time, and a better knowledge of the pathological chemistry of disturbances of, particularly, proteid metabolism may add to this number.

Already Förster ('65) has advanced the hypothetical view that chemical alteration of the maternal blood may be one of the causes underlying the origin of monsters. This view although —strangely enough—almost entirely overlooked is now, however, not insupportable. For, recent advances in pathological chemistry have made us familiar with the chemical changes which our organism is subject to under certain pathological conditions. At the same time a better insight has been gained into the close physiological relation existing between the developing embryo and the mother. Thus, in the light of our present knowledge

we must admit that Förster's idea although at the time expressed in a rather vague manner, is characterized by great foresight. One gains this impression particularly from Wolff's ('13) excellent summary on the biological relationship between embryo and mother during pregnancy.

This relation is, according to Wolff, particularly intimate in man, for the nutrition of the embryo is directly from the mother's blood. It is also of especial interest in such cases where, owing to pathological conditions, some toxic products of metabolism accumulate in the mother's blood. That the embryo is influenced by such alterations of maternal blood, is, according to this author, very plainly evidenced by clinical as well as experimental pathology. Thus it has, for instance, been found that the fetus of a nephritic mother may suffer from oedema and ascites (Sitzenfrey '10). The same obtains also in the animal experiment, if pregnancy is induced in a nephrotomized female.

As a very interesting illustration of the intimacy of physiological relation between fetus and mother in man may be considered the observations recorded by Kehrer.[9] According to this author bile acids and bile pigments can in the animal experiment not cross the placental barrier and thus they never reach the embryo, while in man the observation can be made that children of women suffering from jaundice during pregnancy may have jaundice at birth.

Such data would seem to leave no doubt that the mammalian embryo is sometimes subject to influences of a disturbed maternal metabolism. Whether all products of a deranged metabolism will have a deleterious effect on the embryo is a matter which, of course, needs investigation. In the preceding I have shown that if two substances known to occur in disturbances or carbohydrate metabolism, viz. butyric acid and acetone, be allowed to act on fertilized teleost eggs, the latter will develop into various monsters very strikingly resembling the monsters of man and other mammals. Even teratomata have thus been produced. These results would seem to leave little room for doubt that mammalian monsters may often be due to a coinci-

[9] Quoted from Wolff ('14).

dence of pregnancy with at least such disturbances of metabolism as diabetes.

This coincidence has been found to be fatal to the embryo. Thus, according to Seitz ('13) diabetes melitus has for a long time been considered as causing sterility in women. It is quite possible, I think, that in such individuals the ovum, due to toxic changes, might be expelled soon after conception, that such early abortions might have been mistaken for menstruation and that while perhaps no sterility existed a similar effect has been mistaken for it.

For, to quote Seitz:

. . . . erst als Duncan über mehrere Fälle von Gravidität bei Zuckerkrankheit berichtete, änderten sich die Ansichten und nach den vorliegenden Statistiken über insgesamt 427 Frauen in geschlechtsfähigen Alter darf man annehmen, dass bei rund 5 procent der diabetischen Frauen Schwangerschaft eintritt.

The influence of diabetes on the development of the offspring is according to Seitz very disastrous:

Besonders ungünstig ist die Rückwirkung des Diabetes auf des *Kind.* Übereinstimmend berichten die Statistiken, dass rund 50 procent aller Kinder intrauterin zugrunde gehen und zwar kann der Tod des Kindes jederzeit eintreten, sowohl in den ersten Monaten der Schwangerschaft als auch noch gegen Ende. . . . . Ein weiterer Teil der Kinder wird frühzeitig und schwach geboren; wiederholt wurde auch Erkrankung an Polyurie, an kongenitalem Diabetes und an Hydrocephalus beobachtet.

The data quoted above are, obviously, insufficient and, coming, as they do, from clinicians, they leave us altogether in the dark regarding the morphological effects of maternal diabetes on the embryo. The desirability of comprehensive data regarding fetuses aborted by diabetic mothers as well as those suffering from other diseases of metabolism can not be urged enough upon physicians, as they may not infrequently chance to observe these experiments of nature. But even more desirable would seem to be experiments on mammals. Here the various pathological conditions of metabolism must be imitated by experiment as closely as possible, and the animals so diseased mated in

various combinations. The difficulties of such investigations are numerous, even if the most important one did not exist, namely that the experimental pathology of metabolism is practically yet in its infancy. But such difficulties may perhaps in time be at least partially overcome. And while the complete solution of the problem of the causal factors underlying the origin of monsters may yet be distant, well planned experiments and careful analysis of results may at least furnish direct evidence for the correctness of our assumption that monstrous development is primarly due to parental metabolic toxaemia, an hypothesis which in view of the noted results of our experiments would seem to be well justified.

Our experiments have, besides, pointed out the direction for investigations into the morphogenesis of monsters. In the preceding pages I have shown that microscopic analysis of all monsters discloses evidence of an action on the egg treated with butyric acid and acetone, which tends to dissociate (or disrupt) the germ's substance. This action, blastolysis, is a complex component in the sense of Roux ('95), being the result of the collective action of a number of factors potent in a varying degree in all eggs.

Some observations made in experiments during the summer of 1915 seem to suggest that the most important direct factors whose action results in blastolysis are the toxic effect of the chemical modification of the environment and an increase in osmotic pressure mainly after transfer of the eggs to pure sea-water. The first materially alters or destroys parts of the germ, while the latter may be regarded as a very forceful dissociating agent which disrupts the injured germ. The alteration brought about by the action of butyric acid or acetone seems to be due to the solvent action of these acids. To these conclusions would seem to point the fact that some hours after transfer of the eggs from butyric acid or acetone into pure sea-water there could invariably be found a sediment at the bottom of the dish in which they were kept.

The sediment was of a slimy consistency in the dishes containing eggs which had been treated with butyric acid, while

in the dishes into which acetone-treated eggs had been transferred the sediment made the impression of a granular precipitate. Chemical analysis of these sediments may eventually disclose the nature of the chemical alteration of the eggs treated with these organic acids.

One rather evident effect which they have on the egg is that they increase its permeability. For on transfer from the solution to pure sea-water the eggs, owing to increased imbibition of sea-water, swell to an unusual size: after some time, however, they return to approximately the normal size and occasionally some eggs may be found considerably below the size of the normal, untreated egg. This indicates that the increase in permeability has called forth an increase in endosmotic pressure (imbibition) which allowed enough sea-water to enter the egg to dilute the substances dissolved by the acids, and a subsequent increase in (ex)osmotic pressure (shrinkage) owing to which the dissolved substances have passed out of the eggs and formed the sediment on the bottom.

Besides these two factors (toxicity of the medium and osmotic pressure) which are directly concerned with blastolysis there seem to be some other factors, to which apparently is due the enormous variation in the blastolytic effect and consequently the variation in the morphological deviations from the typical development. While some observations have been made which may bear on the nature of these factors, I can make no definite suggestion at this time and the determination of these factors must be deferred to future experiments.

However, while the analysis of blastolytic action into its component factors must thus for the present time remain incomplete, the evidence of this action is indeed very striking in practically all deformed embryos of my experiments.

Considering the fact that all these terata have been produced by experimentally induced blastolysis from eggs which would in a normal environment have given rise to normal embryos, it would no longer seem to be necessary to assume 'germinal variation' as the cause underlying the origin of ophthalmic monsters and various duplicate twins, as this has been postulated by

some authors and notably by v. Hippel ('09) and H. H. Wilder ('08). The latter author, led by the "symmetry and regularity in anatomical details" of some monsters (cyclopia, diplopagus) has come to look upon them as "beings as orderly and perfect in their development as are the usual and normal types of being." In contradistinction to deformed embryos (true monsters) he even proposes the term "Cosmobion (plural cosmobia)" to designate such 'regular' symmetrical monsters, the underlying primary cause of which he assumes to be germinal variation. Quite apart from the circumstance that the cause of 'germinal variation' would still have to be explained, its assumption would seem unnecessary, because it can be demonstrated that of any given batch of eggs of approximately the same (early) stage of development those left in a normal environment will develop into beings typical for their species, while those subjected to the influence of agents which induce blastolysis will develop into monsters of which some may come very near to Wilder's own standard of 'cosmobia.'[10]

In our experiments such monsters have developed under the blastolyzing influence of some products of pathologic metabolism which might be imagined to be acting on the mammalian ovum during its uterine as well as pre-uterine existence. In the latter case we would be presented with what might, in a restricted sense of the word, be called 'germinal variation.' This germinal deviation, however, being due to a pathological cause, it would seem unwarranted to regard the developmental products of such ova, no matter how symmetrical and well formed they might be, as 'cosmobia' ('orderly living beings'). Besides, it is not entirely improbable that true 'cosmobia' might be produced, if it only were possible to imitate exactly the environmental modifications which underlie the origin of monsters in nature. This degree of accuracy is, however, not yet attained in our experiments.

[10] One cannot but admire the refinement of Wilder's morphological speculations. But, with all due respect for this accomplished morphological philosopher, it is difficult to accept his theory of cosmobia. For, being, as it is, of necessity based on the assumption of germinal variation (of an apathological nature) it leads to the pessimistic conclusion that the problem of the origin of monsters (or at least of 'symmetrical monsters'—'cosmobia') is beyond control.

The assumption of the action of parental metabolic toxaemia on the (uterine or pre-uterine) ovum might also reasonably be extended to the male germ cell, which in fertilizing a normal ovum might cause it to develop into a monster. In this case the chemically altered spermatozoon would probably act in a manner very similar to the germ cell of another species. For, as it will be remembered, Moenkhaus ('04) has demonstrated that if eggs of Fundulus heteroclitus be fertilized with the sperm of Menidia various deformities will result. This experiment has since been repeated by Loeb ('15) and myself[11] and most recently by Reagan and Thorington ('15). Foreign sperm has evidently a toxic effect on the egg and thus deranges its development from the typical course. In analogy, the same may be true for the chemically altered spermatozoon of the same species. That thus modified spermatozoa, if fertilizing normal ova, may cause them to develop into degenerate individuals appears to be probable from statistical data on children of male alcoholics (Stockard '12). Very recently Stockard ('14) has demonstrated that the progeny of experimentally alcoholized guinea pigs, which had been mated to a normal female, was degenerate and deformed, thus suggesting that the chemical injury sustained by the chromatic substance of the spermatozoa had a deleterious effect on the normal ova of healthy females. Since male individuals are just as subject to disturbances of metabolism (although possibly less frequently) it is not improbable that the sperm of males suffering from metabolic toxaemia may bring about abnormal development when fertilizing normal ova. The effects of this union of normal chromatin with chemically altered chromatin may even be unnoticeable in the first generation of offspring and appear in the second and further consecutive generations as in the above mentioned breeding experiments of Stockard with guinea pigs. This author even reports that the results of mating descendants from an alcoholized father and a healthy mother deteriorate markedly with each consecutive generation. These data are very important for our considerations. For, if toxic

[11] Not published.

products of pathologic metabolism should have an injurious influence on the male sex-cells—and there is every reason to believe that they do have such an effect—the chance for a teratogenic influence of parental metabolic toxaemia on the offspring is greatly enhanced.

Elsewhere I (Werber '15 b) have pointed out the bearing which these conclusions and the results of my experiments with products of metabolic toxaemia on the teleost ovum may have on some, so far elusive, problems of medicine. Not less evident is their possible significance for eugenics and the biology of the race.

From the point of view, however, of the embryologist and pathologist our hypothesis and the results of the present experimental study based on it would seem to offer a rational basis for the solution of the old problems of the etiology and the morphogenesis of terata occurring spontaneously in many animals and notably in man and other mammals.

These problems may, I think, now be, at least partly accessible to experimental control.

V. SUMMARY

1. Recent results of investigations in experimental embryology and teratology pointed to the conclusion that the primary causes underlying the origin of monsters in man and other mammals are of a chemical nature. They also suggested that in the latter, particularly, these striking deviations from the developmental norm are due to autogenous chemical modification of the parental blood during disturbances of metabolism.

2. On the basis of this hypothesis experiments were performed on fertilized eggs of Fundulus heteroclitus which were subjected to the action of some substances of certain metabolic toxaemias.

3. Positive results were obtained with particularly two substances, which occur in toxaemia due to disturbances of carbohydrate metabolism, namely butyric acid and acetone.

4. A very great variety of monsters has resulted from these experiments, analogous to human and other mammalian monsters. The deformities concern the eyes (cyclopia, synophthal-

mia, monophthalmia asymmetrica, and anophthalmia), the ear vesicles (rudimentary structure, or lacking, or presence of one vesicle only, or synotia), the olfactory pits, the mouth, the central nervous system, the heart and blood vessels, the fins (unpaired pectoral fins, absence of pectoral fins or all fins, club-tail, etc.), and body form.

5. Oedematous conditions were found in many embryos lacking a continuous system of blood circulation in various parts of the body, which were greatly distended and contained plasma or fibrin and, not infrequently, many lymphocytes. This condition of hydrops is most frequently found in the head. It may be intracerebral or extracerebral and suggests an analogy with the congenital internal and external hydrocephalus of man, which latter may perhaps also be due to developmental imperfections of the blood-vascular system.

6. Blastolytic action of the chemically modified environment is assumed as a morphogenetic principle common to all terata of these experiments. Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter.

7. The nature of blastolysis is two-fold, namely chemical and osmotic.

a. *Chemical blastolysis is a process of chemical alteration (by solvent or precipitating or coagulating action) of the germ's substance. This alteration results in dissociation or disintegration of parts of the latter (defect), or, occasionally in a decrease of the germ's chemical capacity for development and differentiation (inhibition).*

b. *Osmotic blastolysis sets in while the eggs are under the influence of the toxic solutions employed and again (more so) on their transfer from these solutions to pure sea-water. It results from the increase of permeability which allows sea-water to enter the eggs. The imbibition of sea-water by the eggs which swell rapidly, calls forth a fragmentation of the germ and dispersion of its parts which at this stage are yet capable of further independent development and differentiation.*

8. *All eye terata (cyclopia, synophthalmia, monophthalmia lateralis, anophthalmia) are due to a defect, viz., to blastolytic elimination*

of a fragment of, either, ophthalmoblastic or potential interocular material and not to an inhibition as was held by Huschke and Dareste and is now postulated by Stockard.

Only such cases of anophthalmia, where on microscopic examination rudiments (ill-differentiated optic vesicles or cups) are found, form an exception to this rule. Here an inhibition is assumed, due to a decrease of the chemical capacity for development (chemical exhaustion).

9. The frequent occurrence in these experiments of terata of the eyes (or the anterior part of the head) only is regarded as being due to the highest degree of susceptibility of that part of the earliest embryonic primordium, which eventually becomes the embryo's anterior end (animal pole). This assumption supported by many data, is based on Child's discovery of a definite susceptibility gradient ('metabolic gradient'-Child) along the chief body axis of many animals from various phyla.

10. The numerous meroplasts recorded and especially such teratomata as the 'solitary eye' and the 'isolated eye' point to a very high degree of capability of parts of the embryonic primordium for independent development and differentiation (self-differentiation—Roux).

11. While the occurrence in these experiments of various duplicities would seem to point to a relatively high prospective potency of parts of the teleost egg, the latter decreases very rapidly, for at an early stage (the Randwulst-stage or thereabout) these parts are already specifically predetermined as early (undifferentiated) anlagen of some organs. If at this stage a fragment be eliminated the result will be a defect of a corresponding organ or part of the body. Accordingly the teratogenetic time limit must be regarded as very brief, and especially so in the case of duplicities.

12. The results obtained tend to justify the hypothesis on which the experiments were based, namely that parental metabolic toxaemia may be the cause, or, at least, the chief cause underlying the origin of monsters.

DECEMBER 24, 1915

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PLATE 1

EXPLANATION OF FIGURES

63 Photomicrograph of a transverse section through the eyes and forebrain of the embryo in figure 3. *fbr.*, unilobed small forebrain; *o.p.*, fused olfactory pit. × 116.

64 Photomicrograph of a more posterior section (region of optic lobes) of the same embryo. *o.l.*, optic lobes; *o.c.*, optic cross. × 116.

65 Photomicrograph of a transverse section through the eye of the cyclopean embryo in figure 5. *o.l.*, optic lobes. × 135.

66 Photomicrograph of a transverse section through the eye of the cyclopean embryo in figure 12. *fbr.*, forebrain; *o.c.f.*, blastolytic optic cup fragment. × 116

67 A more posterior section (midbrain) of the same embryo, to demonstrate the large size of the fused eye and the small size of the defective midbrain. *mb.*, midbrain; *e.c.*, ear cartilage; *m.*, mouth. × 116.

68 Photomicrograph of a transverse section through the anterior part of the eye of the cyclopean embryo in figure 6. *p.*, pigment epithelium, *r.c.*, rods and cones; *o.p.*, olfactory pit; *br.*, brain. × 116.

69 A more posterior section through embryo in figure 6. *m.o.*, medulla oblongata; *oe.*, oedema; *pl.*, plasma of pericardium, into which the eye dips; *p.*, pigment epithelium; *r.*, retina. × 116.

70 Photomicrograph of a transverse section through the eye of the cyclopean embryo in figure 8, *fbr.*, forebrain; *y.*, yolk. × 116.


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PLATE 2

EXPLANATION OF FIGURES

71 Section through the region of the ear vesticles of the embryo in figure 8. The semicircular canals are on both sides all fused into one, *m.o.*, medulla oblongata; *ph.*, pharynx. × 116.

72 Photomicrograph of a part of a transverse section through the head of the embryo in figure 13 showing three small fused olfactory pits *o.p.* on the deformed side, *o.c.*, oral cavity. × 116.

73 A section more posterior of the same embryo. *n.e.*, the last part of the normal eye; *r.e.*, rudimentary eye; *o.p.*, one of the three olfactory pits on the abnormal side; *o.l.*, optic lobe. × 116.

74 Photomicrograph of a transverse section of the embryo in figure 13, more posterior to the section in figure 73. *s.c.c.*, semicircular canals; *o.l.*, optic lobe; *o.l.f.*, optic lobe fragment; *h.*, heart. × 116.

75 A transverse section of the embryo in figure 17 through the region of the ear vesicles, *h.e.*, heterotopic eye; *s.c.c.*, semicircular canals; *h.*, heart; *l.c.*, lymphocytes; *y.s.*, yolk-sac. × 116.

76 Photomicrograph of a transverse section through the posterior region of the eye of the embryo in figure 16. *o.c.f.*, blastolytic optic cup fragments; *o.c.*, oral cavity. × 116.

77 Photomicrograph of a transverse section through the eye region of the embryo in figure 15. *o.c.f.*, blastolytic optic cup fragment; *o.c.*, oral cavity. × 116.

78 Photomicrograph of a transverse section of the embryo in figure 14, showing one normal eye, a dislocated olfactory pit, *o.p.*, and an 'independent' lens, *l.* on the maxilla, *m.*, mouth. × 90.


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PLATE 3

EXPLANATION OF FIGURES

79 A section posterior to the one in figure 78. *l.*, small independent lenses; *o.p.*, olfactory pit; *m.*, mouth. × 90.

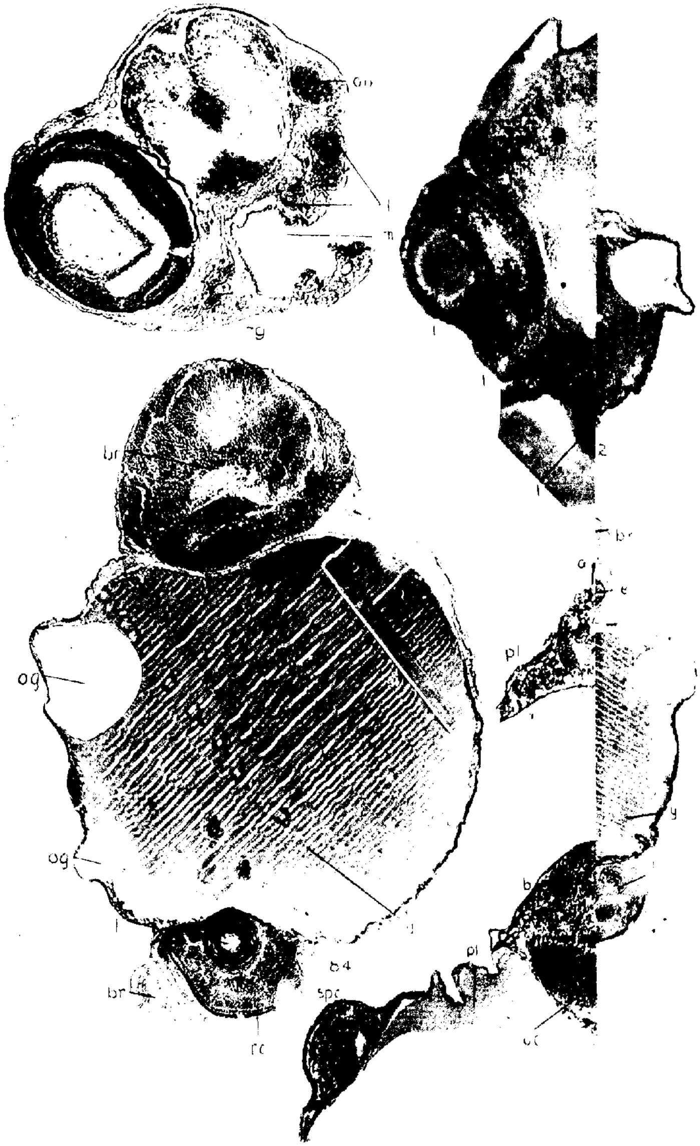
80 Photomicrograph of a transverse section through the anterior part of the head of the embryo in figure 20. *br.*, brain; *l.*, lenses; *y.*, yolk. × 135.

81 Photomicrograph of a sagittal section through the meroplast in figure 38. *br.*, brain fragment; *l.*, lens; *r.c.*, rods and cones; *l.c.*, lymphocytes; *y.*, yolk; *pl.*, plasma. × 64.

82, 83, 84 Photomicrographs of transverse sections through embryo with 'isolated eye' (fig. 42), *br.*, brain; *e.*, eye; *l.*, lens; *y.*, yolk; *r.c.*, rods and cones; *o.g.*, oil globules. Figures 82 and 83 × 70. Figure 84 × 80. (The right side of figures 82 and 83 corresponds to the left side of figure 84; due to reversion of preparation in photographing.)

85, 86, 87 Photomicrographs of transverse sections through amorphous embryo in figure 30, *a*, brain component a; *b*, brain component b; *l.*, lens; *pl.*, plasma; *i.*, intestine; *o.c.*, optic cup; *c.d.*, notochord; *sp.c.*, spinal cord; *y.*, yolk. × 116.

88 and 89 Photomicrographs of transverse sections through amorphous embryo in figure 31, *e.v.*, ear vesicles; *m.*, muscles; *i.*, intestine; *f.*, fin; *pl.*, plasma in oedematous cavity of fin; *y.*, yolk. × 120.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